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(54) Sustained-release preparation of anti-endothelin substance.

(57) A sustained-release preparation containing an anti-endothelin substance and a biodegradable polymer.

The sustained-release preparation of the present invention sustainedly releases an anti-endothelin substance, serving well in the treatment of endothelin-associated diseases.

EP 0 647 449 A1

FIELD OF THE INVENTION

The present invention relates to a sustained-release preparation of an anti-endothelin substance, such as an endothelin antagonist, used to treat endothelin-associated diseases, particularly chronic diseases, such as chronic complications in diabetes mellitus.

BACKGROUND OF THE INVENTION

Showing various potent physiological actions, peptides have been applied as pharmaceuticals in numerous attempts. Their biological half-life, however, is usually very short; for a sustained pharmacologic effect, peptides must be frequently administered, resulting in severe suffering by the patient. Endothelin, a peptide secreted by the vascular endothelium, shows vascular smooth muscle constricting action, both potent and sustainable. Endothelin is therefore important both physiologically and pathologically. Also, there have been reports of the development of peptide-based endothelin antagonists, with the strong expectation that anti-endothelin substances such as endothelin receptor antagonists will contribute to the treatment of various diseases associated with endothelin. For the reasons described above, however, the application of such peptide-based antagonists as pharmaceuticals has been limited. Also, in therapeutic application of conventional endothelin antagonists, there have been attempts to prevent the onset and progress of pathologic states by antagonizing endothelin-associated reactions in acute diseases such as attacks and shocks of acute myocardial infarction. Although application to the treatment of hypertension, cardiac/cerebral circulatory diseases, renal diseases and other diseases has been suggested, there is no specific exemplification. Nor has there been any finding that administration of endothelin antagonists is effective in preventing the onset and progress of endothelin-associated pathologic states in chronic diseases such as diabetic nephropathy.

Various sustained-release preparations are known, including the release rate controlling system based on a polymeric matrix containing a polypeptide dispersed in a poly(lactide-glycolide) copolymer, described in Japanese Patent Unexamined Publication No.2930/1988 (EP-A-251476).

Japanese Patent Examined Publication No. 40329/1992 (Japanese Patent Unexamined Publication No. 118512/1982, EP-A-52510) discloses a composition comprising a biodegrable poly(lactide-glycolide) copolymer which is biologically compatible with luteinizing hormone-releasing hormone (LH-RH) or an analog thereof, a water-soluble polypeptide, and which is capable of sustained release of an effective amount of the polypeptide over a period of at least 1 month.

Japanese Patent Unexamined Publication No. 124814/1990 (EP-A-350246) discloses an art in which a water-soluble drug is effectively packed in microcapsules by adding drug retaining substance comprising an organic basic substance such as a basic amino acid and using a wall made of polymer, and excessive drug release just after administration is suppressed.

There is no sustained-release preparation which comprises a combination of an anti-endothelin substance and a biodegradable polymer and which is capable of effective sustained release of the anti-endothelin substance at an almost constant rate.

Against the above background there is a need for an excellent sustained-release preparation for the treatment of chronic diseases caused by endothelin.

SUMMARY OF THE INVENTION

According to the present invention, there is provided:

(1) a sustained-release preparation which comprises an anti-endothelin substance and a biodegradable polymer,

(2) the sustained-release preparation according to (1) above, wherein the anti-endothelin substance is an endothelin antagonist,

(3) the sustained-release preparation according to (2) above, wherein the endothelin antagonist is a peptide,

(4) the sustained-release preparation according to (2) above, wherein the endothelin antagonist is a peptide of the general formula:

$$\text{A-X-Y-B-C-E} \qquad [\text{I}]$$

wherein X and Y independently represent an α-amino acid residue; A represents a D-acidic-α-amino acid residue; B represents a neutral-α-amino acid residue; C represents an L-α-amino acid residue; E represents a D-α-amino acid residue having an aromatic cyclic group, or an ester thereof, or a salt thereof,

(5) the sustained-release preparation according to (4) above, wherein the peptide is a compound of the formula cyclo[-D-Asp-Asp(R1')-Asp-D-Thg(2)-Leu-D-Trp-] wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide; and Thg(2) represents 2-thienylglycine; Leu represents leucine; Trp represents tryptophan,

(6) the sustained-release preparation according to (4) above, wherein A is a D-acidic-α-amino acid residue which is esterified with an alkyl group,

(7) the sustained-release preparation according to (4) above, wherein Y is a L-acidic-α-amino acid residue,

(8) the sustained-release preparation according to (4) above, wherein Y is a L-acidic-α-amino acid residue which is esterified with an alkyl group,

(9) the sustained-release preparation according to (4) above, wherein the peptide is a compound of the formula, cyclo-[-D-Asp(OC_2H_5)-Asp(R1')-Asp(OC_2H_5)-D-Thg(2)-Leu-D-Trp-], wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide, Thg(2) represents 2-thienylglycine; Leu represents leucine; and Trp represents tryptophan,

(10) the sustained-release preparation according to (4) above, wherein the salt is a polyvalent metal salt,

(11) the sustained-release preparation according to (10) above, wherein the polyvalent metal salt is a zinc salt,

(12) the sustained-release preparation according to (1) above, wherein the biodegradable polymer is an aliphatic polyester,

(13) the sustained-release preparation according to (12) above, wherein the aliphatic polyester is a copolymer of glycolic acid and lactic acid,

(14) the sustained-release preparation according to (13) above, wherein the copolymer has a weight-average molecular weight of about 2,000 to 50,000, as determined by Gel Permeation Chromatography,

(15) the sustained-release preparation according to (13) above, wherein the copolymer has a dispersity of about 0.2 to 4.0,

(16) the sustained-release preparation according to (1) above, which further comprises an organic basic substance,

(17) the sustained-release preparation according to (1) above, which further comprises a water-soluble polyvalent metal salt,

(18) the sustained-release preparation according to (1) above for treatment of diseases caused by endothelin,

(19) the sustained-release preparation according to (18) above, wherein the diseases are chronic diseases,

(20) the sustained-release preparation according to (19) above, wherein the chronic diseases are chronic complications in diabetes mellitus,

(21) the sustained-release preparation according to (20) above, wherein the chronic complications are diabetic nephropathy,

(22) an injectable preparation which comprises the sustained-release preparation according to (1) above.

(23) a peptide of the general formula:

$$\text{A'-X-Y-B-C-E} \qquad \text{[II]}$$

wherein X and Y independently represent an α-amino acid residue; A' represents a D-acidic-α-amino acid residue which is esterified with an alkyl group; B represents a neutral-α-amino acid residue; C represents an L-α-amino acid residue; E represents a D-α-amino acid residue having an aromatic cyclic group, or a salt thereof,

(24) the peptide according to (23) above, wherein X is an L-isomer,

(25) the peptide according to (23) above, wherein Y is an L-isomer,

(26) the peptide according to (23) above, wherein A' is D-glutamic acid or D-aspartic acid which is esterified with an alkyl group,

(27) the peptide according to (23) above, wherein B is an D-isomer,

(28) the peptide according to (23) above, wherein B is selected from the group consisting of D-leucine, D-alloisoleucine, D-tertiary leucine, D-gamma methyl leucine, D-phenylglycine, D-2-thienylglycine, D-3-thienylglycine, D-2-cyclopentylglycine, D-phenylalanine, D-2-thienylalanine, D-valine, D-2-furylglycine and D-3-furylglycine residues,

(29) the peptide according to (23) above, wherein C is selected from the group consisting of L-leucine, L-phenylalanine and L-tryptophan residues,

(30) the peptide according to (23) above, wherein E is selected from the group consisting of D-tryptophan

or derivatives thereof, D-1-naphthylalanine, D-2-naphthylalanine, D-benzothienylalanine, D-4-bisphenylalanine and D-pentamethyl phenylalanine residues,

(31) the peptide according to (23) above, wherein Y is an α-amino acid residue having a carboxyl group which is esterified with an alkyl group,

(32) a peptide according to the formula: cyclo-[-D-Asp(OC_2H_6)-Asp(R1')-Asp(OC_2H_6)-D-Thg(2)-Leu-D-Trp-], wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide; Thg(2) represents 2-thienylglycine; Leu represents leucine; and Trp represents tryptophan, or a salt thereof, and

(33) a zinc salt of a peptide represented by the general formula:

$$\text{A-X-Y-B-C-E} \qquad [\text{I}]$$

wherein X and Y independently represent an α-amino acid residue; A represents a D-acidic-α-amino acid residue; B represents a neutral-α-amino acid residue; C represents an L-α-amino acid residue; E represents a D-α-amino acid residue having an aromatic cyclic group.

As the pathologic state of diabetics is better managed as a result of advances in medicine and pharmacology, the life span of diabetics is increasing also. The extended period of the diabetic condition, however, has raised the problem of chronic complications, especially vascular disorders. Vascular disorders are known to cause various organ disorders because the former occur in coronary arteries, cerebral arteries and microvessels such as those in the retina and renal glomeruli. An example of a chronic complication in diabetes mellitus is nephropathy. Although many factors have been suggested as being involved in the onset of diabetic nephropathy, mesangial thickening and mesangial cell proliferation are marked pathologic factors. It has been assumed that such mesangial thickening eventually destroys glomeruli, causing terminal renal failure. Endothelin, secreted from vascular endothelial cells, is known to be released in large amounts from damaged vessels. Based on the fact that in mesangial cells endothelin stimulates various reactions associated with cell proliferation, such as thymidine uptake, Na^+/H^+ exchange and c-fos expression, the possibility is suggested that chronic exposure to excess endothelin can be the initial stimulation to cause mesangial cell proliferation, suggesting the involvement of endothelin in diabetic nephropathy. In complications other than nephropathy (e.g., diabetic cardiomyopathy and diabetic retinopathy) as well, endothelin resulting from vascular disorders may be involved in the chronic fixation of the pathologic state. Also, since arteriosclerosis and hyperlipidemia are often seen in diabetes mellitus, with some disorder of endothelial cells, involvement of endothelin in these pathologic states is suspected. There are other endothelin-associated diseases, particularly chronic ones, whose onset and progress can be prevented by applying the therapy of the present invention for sustained retention of an anti-endothelin substance in the living body.

DETAILED DESCRIPTION OF THE INVENTION

Where amino acids are expressed by abbreviations, the abbreviations recommended by IUPAC-IUB Commission on Biochemical Nomenclature (European Journal of Biochemistry 138, 9-37, 1984) or the abbreviations in common usage in the art are used. Where optical isomers exist for any compound, the L-isomer is meant unless otherwise indicated.

In the present invention, the anti-endothelin substance is exemplified by antibodies against endothelin, antibodies against endothelin receptors, high molecular substances represented by soluble endothelin receptors, endothelin antagonists obtained by chemical synthesis or fermentation, and substances which inhibit endothelin production (endothelin converting enzyme inhibitors).

The anti-endothelin substance in the present invention inhibits the binding of endothelin to its receptors. For example, the anti-endothelin substance inhibits the binding of endothelin-1 to a membrane fraction prepared from a homogenate of swine aortic smooth muscle. It is reported that there are at least two subtypes of endothelin receptors, referred to as ET-A and ET-B, respectively. The anti-endothelin substance in the present invention antagonizes one or both of these two receptors.

The anti-endothelin substance in the present invention inhibits vascular or muscular contraction induced by endothelin-1 administration in spiral specimens of swine coronary artery with the endothelial cells removed, specimens of the excised guinea pig tracheal muscle or specimens of the excised swine cerebral basal artery, antagonizes the increase in perfusion pressure by endothelin in excised rat hearts, and improves mortality in mice receiving endotoxin.

The anti-endothelin substance in the present invention may be water soluble or oil soluble. The degree of water solubility in the present invention is preferably octanol/water ratios of not higher than 0.1. The degree of oil solubility in the present invention is preferably octanol/water ratios of over 0.1. Also, the anti-endothelin

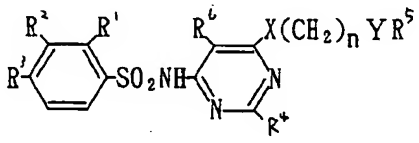
substance may be soluble in polar solvents such as acetonitrile, dichloromethane and chloroform at not less than 10 mg/ml and not more than 100 mg/ml. It may also be almost insoluble in acetonitrile, dichloromethane and chloroform.

In the present invention, the anti-endothelin substance is preferably an endothelin antagonist, as exemplified by non-peptide compounds, peptides and derivatives thereof obtained by chemical synthesis or fermentation, peptides and derivatives thereof. Here, the peptide may be a chain or cyclic peptide or a cyclic and chain peptide.

Examples of non-peptide compounds include the non-peptides described in European Patent Publication Nos. 510526 and 526708 and WO93/08799.

(1) EPA-510526

A compound represented by the formula



wherein

R^1: a hydrogen atom, lower-alkyl group, lower-alkoxy group, lower-alkylthio group, a halogen atom or trifluoromethyl;

R^2: a hydrogen atom, a halogen atom, lower-alkoxy group, hydroxy-lower-alkoxy group, or trifluoromethyl;

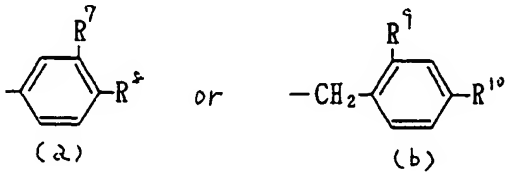
R^3: a hydrogen atom, hydroxy group, a halogen atom, alkylthio group, cycloalkyl group, hydroxy-lower-alkyl group, hydroxy-lower-alkoxy group, hydroximino-lower-alkyl lower-alkenyl group, oxo-lower-alkyl group, trifluoromethyl, trifluoromethoxy, lower-alkoxy group, lower-alkoxy-lower-alkoxy group, aryl-lower-alkoxy group;

R^2 and R^3: together to form butadienyl;

R^4: a hydrogen atom, lower-alkyl group, aryl group or heteroaryl group;

R^5: a hydrogen atom, lower-alkanoyl group, benzoyl, hetrocyclylcarbonyl group, or tetrahydropyran-2-yl;

R^6 is represented by the formula (a) or (b)



(a) or (b)

R^7: a hydrogen atom, lower-alkoxy group or nitro, and R^8 represents a hydrogen atom, a halogen atom, lower-alkyl group, lower-alkoxy group, lower-alkylthio group, nitro, hydroxy, amino or trifluoromethyl;

R^7 and R^8: together to form butadienyl;

R^9: a hydrogen atom, a halogen atom, lower-alkyl group, lower-alkoxy group, lower-alkylthio group or trifluoromethyl;

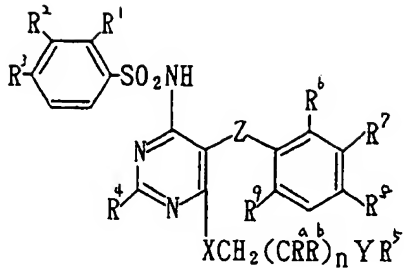
R^{10}: a hydrogen atom, a halogen atom, lower-alkyl group, lower-alkoxy group or lower-alkylthio group;

X and Y: independently O, S or NH;

n: 2, 3 or 4; or a salt thereof;

(2) EPA-526708

A compound represented by the formula



wherein

R^1: a hydrogen atom, lower-alkoxy group, lower-alkylthio group, a halogen atom or trifluoromethyl;

R^2: a hydrogen atom, a halogen atom, lower-alkoxy group, trifluoromethyl or $-OCH_2COOR^a$;

R^3: a hydrogen atom, a halogen atom, lower-alkyl group, lower-alkylthio group, cycloalkyl group, lower-alkoxy group or trifluoromethyl

R^2 and R^3: together to form butadienyl, methylenedioxy, ethylenedioxy or isopropylidendioxy;

R^4: a hydrogen atom, lower-alkyl group, cycloalkyl group, trifluoromethyl, lower-alkoxy group, lower-alkylthio group, lower-alkylthio-lower-alkyl group, hydroxy-lower-alkyl group, hydroxy-lower-alkoxy group, lower-alkoxy-lower-alkyl group, hydroxy-lower-alkoxy-lower-alkyl group, hydroxy-lower-alkoxy-lower-alkoxy group, lower-alkylsulfinyl group, lower-alkylsulfonyl group, 2-methoxy-3-hydroxypropoxy, 2-hydroxy-3-phenylpropyl, amino-lower-alkyl group, lower-alkylamino-lower-alkyl group, di-lower-alkylamino-lower-alkyl group, amino, lower-alkylamino group, di-lower-alkylamino group, arylamino group, aryl group, arylthio group, aryloxy group, aryl-lower-alkyl group or heterocyclyl group;

R^5: a hydrogen atom, lower-alkyl group, lower-alkanoyl group, benzoyl, hetrocyclyl-carbonyl group, hetrocyclyl-methyl, or tetrahydropyran-2-yl;

R^6~R^9: a hydrogen atom, a halogen atom, trifluoromethyl, lower-alkyl group, lower-alkoxy group, lower-alkylthio group, hydroxy, hydroxymethyl, cyano, carboxyl, formyl, methyl sulfinyl, methyl sulfonyl, methyl sulfonyloxy, lower-alkoxy carbonyloxy;

R^7: together with R^6 or R^8 to form butadienyl, methylenedioxy, ethylenedioxy or isopropyliedenedioxy;

Z: -O-, -S-, ethylene, vinylene, -CO-, $-OCHR^{10}-$ or $-SCHR^{10}-$;

R^{10}: a hydrogen atom or lower-alkyl group;

X and Y: independently O, S or NH;

YR^5: lower-alkyl sulfinyl or $-OCH_2CH(OR^c)CH_2R^d$;

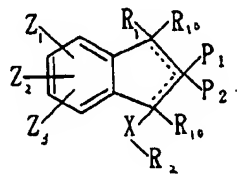
R^a, R^b, R^c and R^d: a hydrogen atom or lower-alkyl group;

R^c and R^d: together to form methylene, ethylene or isopropylidene;

n: 1, 2 or 3; or a salt thereof;

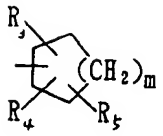
(3) WO93/08799

A compound of formula



wherein:

R_1 is $-X(CH_2)_nAr$ or $-X(CH_2)_nR_8$ or



(c);

R_2 is hydrogen, Ar or (c);

P_1 is $-X(CH_2)_nR_8$;

P_2 is $-X(CH_2)_nR_8$, or $-XR_9Y$;

R_3 and R_5 are independently hydrogen, R_{11}, OH, C_{1-8} alkoxy, $S(O)_qR_{11}$, $N(R_6)_2$, Br, F, I, Cl, CF_3, $NHCOR_6$, $-XR_9-Y$ or $-X(CH_2)_nR_8$ wherein the methylene groups of $-X(CH_2)_bR_8$ may be substituted by one or more $-(CH_2)_nAr$ groups;

R_4 is hydrogen, R_{11}, OH, C_{1-5} alkoxy, $S(O)_qR_{11}$, $N(R_6)_2$, $-X(R_{11})$, Br, F, I, Cl or $NHCOR_6$ wherein the C_{1-5} alkoxy may be substituted by OH, methoxy or halogen;

R_6 is independently hydrogen or C_{1-4} alkyl;

R_7 is independently hydrogen, C_{1-8} alkyl or $(CH_2)_nAr$;

R_8 is hydrogen, R_{11}, CO_2H, PO_3H_2, $P(O)(OH)R_7$ or tetrazole;

R_9 is C_{1-10} alkyl, C_{2-10} alkenyl or phenyl all of which may be substituted by one or more OH, $N(R_6)_2$, COOH, halogen or XC_{1-5} alkyl;

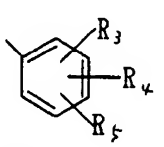
R_{10} is R_3 or R_4;

R_{11} is C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl all of which may be substituted by one or more OH, CH_2OH, $N(R_6)_2$ or halogen;

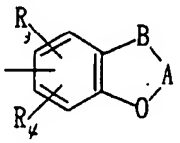
X is $(CH_2)_n$, O, NR_6 or $S(O)_q$;

Y is CH_3 or $-CH_2X(CH_2)_nAr$;

Ar is



(a)



(b)

naphthyl, indolyl, pyridyl or thienyl, oxazolidinyl, oxazolyl, thiazolyl, isothiazolyl, pyrazolyl, triazolyl, tetrazolyl, imidazolyl, imidazolidinyl, thiazolidinyl, isoxazolyl, oxadiazolyl, thiadiazolyl, morpholinyl, piperidinyl, piperazinyl, pyrrolyl, or pyrimidyl; all of which may be substituted by one or more R3 or R4 groups;

A is C = O, or $[C(R_6)_2]_m$;

B is $-CH_2-$ or -O-;

Z_1 and Z_2 are independently hydrogen, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl, OH, C_{1-8} alkoxy, $S(O)_qC_{1-8}$ alkyl, $N(R_6)_2$, Br, F, I, Cl, $NHCOR_6$, $-X(CH_2)_nR_8$, phenyl, benzyl or C_{3-6} cycloalkyl wherein the C_{1-8} alkyl, C_{2-8} alkenyl or C_{2-8} alkynyl may be optionally substituted by COOH, OH, $CO(CH_2)_nCH_3$, $CO(CH_2)_nCH_2N(R_6)_2$, or halogen; or Z_1 and Z_2 together may be -O-A-O- on contiguous carbons;

Z_3 is Z_1 or XR_9Y;

q is zero, one or two;

n is an integer from 0 to six;

m is 1, 2 or 3;

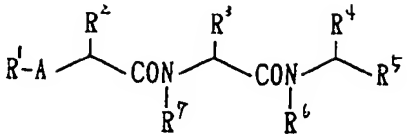
and the dotted line indicates the optional presence of a double bond; or a pharmaceutically acceptable salt thereof; provided that

- R_2 is not hydrogen when X is $S(O)_q$;
- when the optional double bond is present there is only one R_{10} and there is no P_1;
- the compound of Formula I is not (1RS)-1, 3-diphenylindene-2-carboxylic acid; (cis, cis)-(1RS,3SR)-1,3-diphenylindane-2-carboxylic acid; (1RS)-3-[3-Methyl-1-phenyl-(1H)-ind-2-en-1-yl] propionic aicd; or (1RS)-2[1,3-diphenyl-(1H)-ind-2-en-2-yl]ethanoic acid.

Examples of chain peptides include the peptides described in Japanese Patent Unexamined Publication Nos. 244097/1992, 283600/1992 and WO93/10144.

(1) Japanese Patent Unexamined Publication No. 244097/1992

A peptide of the formula:



in which

R^1 is hydrogen or acyl,

R^2 is lower alkyl, optionally substituted ar(lower)alkyl, cyclo(lower)alkyl(lower)alkyl or optionally substituted heterocyclic(lower)alkyl,

R^3 is optionally substituted heterocyclic(lower)alkyl or optionally substituted ar(lower)alkyl,

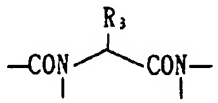
R^4 is hydrogen or optionally substituted lower alkyl,

R^5 is carboxy, protected carboxy, carboxy(lower)alkyl or protected carboxy(lower)alkyl,

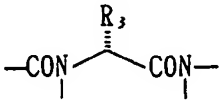
R^6 is hydrogen or optionally substituted lower alkyl,

R^7 is hydrogen or lower alkyl, and

A is -O-, -NH-, lower alkylimino or lower alkylene, provided that when R^2 is (S)-isobutyl, R^3 is N-(dichlorobenzyloxycarbonyl)indol-3-ylmethyl, R^4 is methyl, R^5 is methoxycarbonyl, R^6 is hydrogen, R^7 is hydrogen and A is -NH-, then the partial formula:



has the absolute configuration of



or a pharmaceutically acceptable salt thereof.

(2) Japanese Patent Unexamined Publication No. 283600/1992

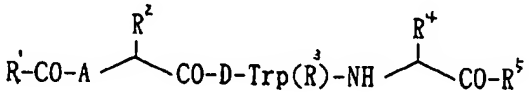
A peptide derivative represented by the formula:

$$R1\text{-}H\ X_1\ D\ X_2\ I\ X_3$$

wherein X_1 represents leucine, arginine or glutamine residue, X_2 represents isoleucine or valine residue, X_3 represents tryptophan, amidotryptophan or D-naphtylalanine residue and R1 represents residual 15 amino acids.

(3) WO93/10144

A compound of the formula:



in which

R^3 is hydrogen or lower alkyl,

R^4 is pyridyl(lower)alkyl; and

R^1 is C_3-C_8 alkyleneamino, N,N-di(lower)alkylamino, N-lower alkyl-N-arylamino, N-lower alkyl-N-C_3-C_8 cycloalkylamino, or C_5-C_{10} bycyclic alkyleneamino,

R^2 is lower alkyl,

R^5 is C_3-C_8 alkyleneamino, N,N-di(lower)alkylamino, morpholino, thiomorpholino, N',N'-di(lower)alkylhydrazino, morpholinoamino, lower alkylpipe razinylamino, lower alkoxy(lower)alkylamino, morpho-

lino(lower)alkylamino, C_3-C_8 alkyleneamino(lower)-alkylamino which may be substituted by oxo, or pyridyl(lower)alkylamino, and

A is lower alkylene; or

R^1 is piperidin-1-yl, lower alkylpiperidin-1-yl, octahydroazocin-1-yl, indolin-1-yl, 1,2,3,4-tetrahydroquinolin-1-yl, N,N-di(lower)alkylamino, N-lower alkyl-N-arylamino, N-lower alkyl-N-C_3-C_8 cycloalkylamino, or C_5-C_{10} bycyclic alkyleneamino,

R^2 is lower alkyl,

R^5 is amino or lower alkylamino, and

A is lower alkylene; or

R^1 is piperidin-1-yl, octahydroazocin-1-yl, N,N-di(lower)alkylamino, or C_5-C_{10} bycyclic alkyleneamino,

R^2 is lower alkyl,

R^5 is amino, lower alkylamino, N,N-di(lower)alkylamino, C_3-C_8 alkyleneamino, or morpholino, and

A is -NH-; or

R^1 hexahydro-1H-azepin-1-yl,

R^2 is isobutyl,

R^5 is ethylamino, and

A is methylene; or

R^1 is N-[1-(dimethylcarbamoyl)-2,2-dimethylpropyl]amino,

R^2 is isobutyl,

R^5 is amino, and

A is -NH-; or

R^1 is N,N-di(lower)alkylamino, 1,2,3,4-tetrahydroquinolin-1-yl, N-lower alkyl-N-arylamino, or N-lower alkyl-N-C_3-C_8 cycloalkylamino,

R^2 is lower alkyl,

R^5 is hydroxy or CO-R^5 is protected carboxy, and

A is lower alkylene; or

R^1 is C_5-C_{10} bycyclic alkyleneamino,

R^2 is lower alkyl,

R^5 is hydroxy or CO-R^5 is protected carboxy, and

A is lower alkylene or -NH-; or

R^1 is N-ethyl-N-(1-ethylpropyl)amino, N-ethyl-N-isopropylamino, N-ethyl-N-neopentylamino, or N-(1-ethylpropyl)-N-propylamino,

R^2 is isobutyl,

R^5 is hydroxy or CO-R^5 is protected carboxy, and

A is -NH-; or

R^1 is piperidin-1-yl,

R^2 is isobutyl,

R^5 is hydroxy or CO-R^5 is protected carboxy, and

A is methylene; or

R^1 is hexahydro-1H-azepin-1-yl,

R^2 is propyl,

R^5 is hydroxy or CO-R^5 is protected carboxy, and

A is -NH-;

or a pharmaceutically acceptable salt thereof.

Examples of cyclic peptides include the peptides described in Japanese Patent Unexamined Publication No. 261198/1992.

Japanese Patent Unexamined Publication No. 261198/1992

A cyclic pentapeptide of the formula:

cyclo (-X^1-X^2-X^3-X^4-X^5-)

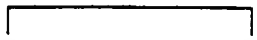
wherein X^1~X^5 represent amino acid residues, respectively, and X^1 is D-Phe, D-Tyr, D-Tha, D-Tza, D-Nal, D-Bta, D-Trp, D-Trp(O), D-Trp(CHO) or D-Trp($(CH_2)_mCOR^1$, wherein m is from 0 to 6, and R^1 is a hydroxyl group, a C_1-C_6 alkoxy group, an amino group or a C_1-C_6 monoalkylamino group, provided that when m=0, R^1 is not a hydroxyl group; X^2 is D-Asp, D-Glu, or D-Cys(O_3H); X^3 is Pro, Hyp, Pip, Thz, β-Ala, Gly, Ala, α-Aba, Aib, Val, Nva, Leu, Ile, alle, Nle, Met, Met(O), Met(O_2), Phe, Tza, Tha, Tyr, Trp, His, Arg, Lys, Lys(CHO), Orn, Orn(O), Asn, Gln, Asp, Glu, Cys(O_3H), Cys, Ser or Thr wherein those α-amino acids having a hydrogen atom on the α-amino group are optionally substituted by a C_1-C_6 alkyl or C_3-C_7 cycloalkyl group which optionally has a group selected from the group consisting of an imidazolyl group, a carboxyl group, a sulfo group and a hydroxy group; X^4 is D-Ala, D-Thr, D-α-Aba, D-Val, D-Nva, D-Leu, D-Ile, D-alle, D-Nle, D-tert-Leu, D-Cpg, D-Chg, D-Dpg, D-

Pen, Aib, Ac_3c, Ac_4c, Ac_5c, Ac_6c, Ac_7c, D-Phg, D-Thg, D-Fug, D-Tzg or D-Itg wherein those α-amino acids having a hydrogen atom at the α-position are optionally substituted by a C_1-C_3 alkyl group; X^5 is Pro, Pip, Thz, His, Ala, α-Aba, Val, Nva, Leu, Ile, alle, Nle, Met, C_3al, C_4al, C_5al or C_6al wherein those α-amino acids having hydrogen atom on the α-amino group are optionally substituted by a C_1-C_6 alkyl group; or a pharmaceutically acceptable salt thereof.

Examples of cyclic and chain peptides-containing compounds include the peptides described in Japanese Patent Unexamined Publication No. 288099/1992.

Japanese Patent Unexamined Publication No. 288099/1992

A peptide represented by the formula

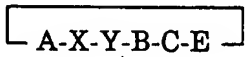


wherein Xaa_1 represents Tyr, Phe or Ala, Xaa_2 represents Asp or Gly, Xaa_3 represents Trp or Phe.

The above-described endothelin antagonists include those produced by microbes, such as cochinmicins, a cyclodepsipeptide [The Journal of Antibiotics, Vol. 45, No. 11, 1709-1722 (1992)].

Examples of endothelin antagonists which antagonize both receptors ET-A and ET-B include the cyclic peptide (I) described hereinafter which is described in European Patent Publication No. 528312 and Japanese Patent Application No. 278722/1993.

More specifically, the anti-endothelin substance in the present invention is preferably a peptide represented by the general formula:

 [I]

wherein X and Y independently represent an α-amino acid residue; A represents a D-acidic-α-amino acid residue; B represents a neutral-α-amino acid residue; C represents an L-α-amino acid residue; E represents a D-α-amino acid residue having an aromatic cyclic group.

With respect to general formula [I], the parent amino acid for the α-amino acid residue represented by X or Y may be any amino acid, as long as it is an α-amino acid. Such amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, 2-aminomalonic acid, 2-aminoadipic acid, glycine, histidine, isoleucine, leucine, lysine, ornithine, 2,4-diaminobutyric acid, methionine, phenylalanine, proline, 4-hydroxyproline, thioproline, azetidine-2-carboxylic acid, pipecolic acid (piperidine-2-carboxylic acid), indoline-2-carboxylic acid, tetrahydroisoquinoline-3-carboxylic acid, serine, threonine, tryptophan, 5-methyltryptophan, tyrosine, valine, alloisoleucine, norvaline, norleucine, tertiary leucine, gamma methylleucine, phenylglycine, 2-aminobutyric acid, cysteic acid, homocysteic acid, 1-naphthylalanine, 2-naphthylalanine, 2-thienylglycine, 3-thienylglycine, 3-benzothienylalanine, 4-biphenylalanine, pentamethylphenylalanine, 1-aminocyclopropane-1-carboxylic acid, 1-aminocyclobutane-1-carboxylic acid, 1-aminocyclopentane-1-carboxylic acid, 1-aminocyclohexane-1-carboxylic acid and 1-aminocycloheptane-1-carboxylic acid. When these α-amino acids have functional groups (e.g., hydroxyl group, thiol group, amino group, imino group and carboxyl group), the functional groups may be substituted for by a suitable substituent.

Hydroxyl groups which are substituted include C_{1-6} alkanoyloxy (e.g., formyloxy, acetoxy and propionyloxy), C_{4-9} alicyclic carbonyloxy (e.g., cyclopentanecarbonyloxy and cyclohexanecarbonyloxy), C_{7-15} arylcarbonyloxy (e.g., benzoyloxy and 4-methylbenzoxloxy), C_{8-16} aralkylcarbonyloxy (e.g., phenylacetoxy, 2-phenylpropionyloxy, 3-phenylpropionyloxy and diphenylacetoxy), aromatic heterocyclicalkylcarbonyloxy (e.g., indol-2-ylacetoxy and indol-3-ylacetoxy), C_{1-6} alkoxy (e.g., methoxy, ethoxy, n-propoxy and tert-butoxy), C_{3-8} cycloalkoxy (e.g., cyclopentoxy and cyclohexyloxy), C_{6-12} aryloxy (e.g., phenyloxy and 4-methylphenyloxy) and C_{7-15} aralkyloxy (e.g., benzyloxy, phenethyloxy and diphenylmethoxy). α-Amino acids in which hydroxyl group is substituted include o-acetylserine, o-acetylthreonine, 4-acetoxyproline, o-benzoylserine, o-benzoylthreonine, 4-benzoyloxyproline, o-phenylacetylserine, o-phenylacetylthreonine, 4-phenylacetoxyproline, o-ethylserine, o-ethylthreonine, 4-ethoxyproline, o-cyclohexylserine, o-cyclohexylthreonine, 4-cyclohexyloxyproline, o-phenylserine, o-phenylthreonine, 4-phenoxyproline, o-benzylserine, o-benzylthreonine, 4-benzyloxyproline, o-diphenylmethylserine, o-diphenylmethylthreonine and 4-diphenylmethoxyproline.

Thiol groups which are substituted include C_{1-6} alkanoylthio (e.g., formylthio, acetylthio and propionylthio), C_{4-9} alicyclic carbonythio (e.g., cyclopentanecarbonylthio and cyclohexanecarbonylthio), C_{7-15} arylcarbonylthio (e.g., benzoylthio and 4-methylbenzoylthio), C_{8-16} aralkylcarbonylthio (e.g., phenylacetylthio, 2-phenylpropio-

nylthio, 3-phenylpropionylthio and diphenylacetylthio), C_{1-6} alkylthio (e.g., methylthio, ethylthio, n-propylthio and tert-butylthio), C_{3-8} cycloalkylthio (e.g., cyclopentylthio and cyclohexylthio), C_{6-12} arylthio (e.g., phenylthio and 4-methylphenylthio) and C_{7-15} aralkylthio (e.g., benzylthio, phenethylthio and diphenylmethylthio). α-Amino acids in which thiol group is substituted include S-acetylcysteine, S-benzoylcysteine, S-phenylacetylcysteine, S-ethylcysteine, S-cyclohexylcysteine, S-phenylcysteine and S-benzylcysteine.

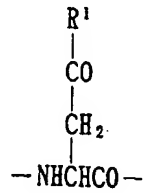
Amino groups which are substituted include C_{1-6} alkylamino (e.g., N-methylamino, N-ethylamino and N-tert-butylamino), C_{3-8} cycloalkylamino (e.g., N-cyclopentylamino and N-cyclohexylamino), C_{6-12} arylamino (e.g., N-phenylamino and N-{4-methyl phenyl}amino), C_{7-15} aralkylamino (e.g., N-benzylamino, N-phenethylamino, N-{2-chlorobenzyl}amino, N-{3-chlorobenzyl}amino, N-{4-chlorobenzyl}amino, N-{2-methylbenzyl}amino, N-{3-methylbenzyl}amino, N-{4-methylbenzyl}amino, N-{2-methoxybenzyl}amino, N-{3-methoxybenzyl}amino and N-{4-methoxybenzyl}amino), aromatic heterocyclic-C_{1-6} alkylamino (e.g., 2-furylmethylamino, 3-furylmethylamino, 2-thienylmethylamino, 3-thienylmethylamino, indol-2-ylmethylamino and indol-3-ylmethylamino), and C_{1-6} aliphatic acylamido (e.g., formamido, acetamido and propionamido), C_{4-9} alicyclic acylamido (e.g., cyclopentanecarboxamido and cyclohexanecarboxamido), C_{7-15} arylacylamido (e.g., benzamido and 4-methylbenzamido), C_{8-16} aralkylacylamido (e.g., phenylacetamido, 2-phenylpropionamido, 3-phenylpropionamido, diphenylacetamido, 1-naphthylacetamido and 2-naphthylacetamido), aromatic heterocycliccarboxamido (e.g., indol-2-ylcarboxamido and indol-3-ylcarboxamido), aromatic heterocyclic-alkylcarboxamido(e.g., indol-2-ylacetamido and indol-3-ylacetamido), and sulfonylamido (e.g., benzenesulfonylamido, p-toluenesulfonylamido and 4-methoxy-2,3,6-trimethylbenzenesulfonylamido). Substituents in imino or imido groups which are substituted are the same as those in each amino or amido groups which are substituted. α-Amino acids wherein the amino group is substituted include N-methylglycine (sarcosine), N-ethylglycine, N-methylleucine, N-ethylleucine, N-methylphenylalanine, N-ethylphenylalanine, N(α)-methyltryptophan, N(α)-ethyltryptophan, N-cyclopentylglycine, N-cyclohexylglycine, N-phenylglycine, N-phenylleucine, N-benzylglycine, N-benzylleucine, N(π)-benzylhistidine, N(τ)-benzylhistidine, N(π)-phenacylhistidine, N(π)-benzyloxymethylhistidine, N^{g}-benzenesulfonylarginine, N^{g}-p-toluenesulfonylarginine,N^{g}-(4-methoxy-2,3,6-trimethylbenzenesulfonyl)arginine, N(ε)-benzenesulfonyllysine, N(ε)-p-toluenesulfonyllysine, N(ε)-(4-methoxy-2,3,6-trimethylbenzenesulfonyl) lysine, N^{in}-methyltryptophan, N^{in}-ethyltryptophan, N^{in}-formyltryptophan, N^{in}-acetyltryptophan, N(ε)-benzyllysine, N(ε)-(2-furylmethyl)lysine, N(ε)-(2-thienylmethyl)lysine, N(ε)-(indol-3-ylmethyl)lysine, N(ε)-phenylacetyl)lysine, N(ε)-({2-furyl} acetyl)lysine, N(ε)-({2-thienyl}acetyl)lysine, N(ε)-({indol-3-yl}acetyl)lysine, N(ε)-benzoyllysine, N(ε)-(3-phenylpropionyl)lysine, N(δ)-benzylornithine, N(δ)-(2-furylmethyl)ornithine, N(δ)-(2-thienylmethyl)ornithine, N(δ)-(indol-3-ylmethyl)ornithine, N(δ)-benzoylornithine, N(δ)-phenylacetylornithine, N(δ)-(3-phenylpropionyl)ornithine, N(δ)-({2-methylphenyl}acetyl))ornithine, N(δ)-({3-methylphenyl}acetyl)ornithine, N(δ)-({4-methylphenyl}acetyl) ornithine, N(δ)-({2-chlorophenyl}acetyl)ornithine, N(δ)-({3-chlorophenyl}acetyl)ornithine, N(δ)-({4-chlorophenyl}acetyl)ornithine, N(δ)-({2-methoxyphenyl}acetyl)ornithine, N(δ)-({3-methoxyphenyl}acetyl)ornithine, N(δ)-({4-methoxyphenyl}acetyl)ornithine, N(δ)-(4-biphenylacetyl)ornithine, N(γ)-benzyl-2,4-diaminobutyric acid, N(γ)-(2-furylmethyl)-2,4-diaminobutyric acid, N(γ)-(2-thienylmethyl)-2,4-diaminobutyric acid, N(γ)-(indol-3-ylmethyl)-2,4-diaminobutyric acid, N(γ)-benzoyl-2,4-diaminobutyric acid, N(γ)-phenylacetyl-2,4-diaminobutyric acid, N(γ)-(3-phenylpropionyl)-2,4-diaminobutyric acid, N(γ)-(2-furylacetyl)-2,4-diaminobutyric acid, N(γ)-(2-thienylacetyl)-2,4-diaminobutyric acid and N(γ)-({indol-3-yl}acetyl)-2,4-diaminobutyric acid.

Carboxyl groups which are substituted include carbamoyl group (-$CONH_2$) and substituted carbamoyl group such as N-C_{1-6} alkylcarbamoyl (e.g., methylcarbamoyl, ethylcarbamoyl, n-propylcarbamoyl and tert-butylcarbamoyl), C_{3-8} cycloalkylcarbamoyl (e.g., cyclopentylcarbamoyl and cyclohexylcarbamoyl), C_{6-12} arylcarbamoyl (e.g., phenylcarbamoyl and 4-methylphenylcarbamoyl), C_{7-15} aralkylcarbamoyl (e.g., benzylcarbamoyl, phenetyl and 1,2-diphenylethylcarbamoyl), aromatic heterocyclic-C_{1-6} alkylcarbamoyl (e.g., 2-{indol-2-yl}ethylcarbamoyl and 2-{indol-3-yl}ethylcarbamoyl), piperidinocarbonyl, piperazincarbonyl, N^4-C_{1-6} alkylpiperazincarbonyl (e.g., N^4-methylpiperazincarbonyl and N^4-ethylpiperazincarbonyl), N^4-C_{3-8} cycloalkylpiperazincarbonyl (e.g., N^4-cyclopentylpiperazincarbonyl and N^4-cyclohexylpiperazincarbonyl), N^4-5 to 7-membered heterocyclic piperazincarbonyl (e.g., N^4-pyridylpiperazincarbonyl, N^4-furylpiperazincarbonyl and N^4-thienylpiperazincarbonyl), N^4-C_{6-12} arylpiperazincarbonyl (e.g., N^4-phenylpiperazincarbonyl and N^4-{4-methylphenyl}piperazincarbonyl), N^4-C_{7-15} aralkylpiperazincarbonyl (e.g., N^4-benzylpiperazincarbonyl, N^4-phenetylpiperazincarbonyl and N^4-{1,2-diphenylethyl}piperazincarbonyl), N^4-{aromatic heterocyclic -C_{1-6} alkyl}piperazincarbonyl (e.g., N^4-[2-{indol-2-yl}ethyl]piperazincarbonyl and N^4-[2-{indol-3-yl}ethyl]piperazincarbonyl), N^4-C_{1-6} aliphatic acylpiperazincarbonyl (e.g., N^4-acetylpiperazincarbonyl and N^4-propionylpiperazincarbonyl), N^4-C_{4-9} alicyclic acylpiperazincarbonyl (e.g., N^4-cyclopentanecarbonylpiperazincarbonyl and N^4-cyclohexane carbonylpiperazincarbonyl), N^4-C_{7-15} arylacylpiperazincarbonyl (e.g., N^4-benzoylpiperazincarbonyl and N^4-{4-me-

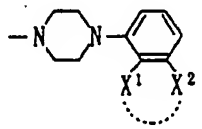
thylbenzoyl}piperazincarbonyl), N^4-C_{8-16} aralkylacylpiperazincarbonyl (e.g., N^4-phenylacetylpiperazincarbonyl, N^4-{2-phenylpropion}piperazincarbonyl, N^4-{3-phenylpropionyl}piperazincarbonyl, N^4-diphenylacetylpiperazincarbonyl, N^4-{1-naphthylacetyl}piperazincarbonyl and N^4-{2-naphthylacetyl}piperazincarbonyl), N^4-{aromatic heterocyclic -carbonyl}piperazincarbonyl (e.g., N^4-{indol-2-ylcarbonyl}piperazincarbonyl and N^4-{indol-3-ylcarbonyl}piperazincarbonyl) and N^4-{aromatic heterocyclicalkylcarbonyl}piperazincarbonyl (e.g., N^4-{indol-2-ylacetyl}piperazincarbonyl and N^4-{indol-3-ylacetyl}piperazincarbonyl), and C_{1-6} alkyloxycarbonyl (e.g., methoxycarbonyl, ethoxycarbonyl and n-propoxycarbonyl), C_{3-8} cycloalkyloxycarbonyl (e.g., cyclopentyloxycarbonyl and cyclohexyloxycarbonyl) and C_{7-15} aralkyloxycarbonyl (e.g., benzyloxycarbonyl, phenethyloxycarbonyl, 1-phenylethoxycarbonyl and diphenylmethoxycarbonyl). The above substituted carbamoyl groups include amides with α-amino acids and amides with oligopeptides (e.g., dipeptide, tripeptide and tetrapeptide). α-amino acids wherein the carboxyl group is substituted include N^4-methylasparagine, N^4-phenylasparagine, N^4-benzylasparagine, N^4-phenetylasparagine, N^4-(2-{indol-3-yl}ethyl)asparagine, N^5-methylglutamine, N^5-phenylglutamine, N^5-benzylglutamine, N^5-phenetylglutamine, N^5-(2-{indol-3-yl}ethyl)glutamine, aspartic acid β-methyl ester, aspartic acid β-cyclopropyl ester, aspartic acid β-benzyl ester, aspartic acid β-phenethyl ester, aspartic acid β-N^4-phenylpiperazinamide, aspartic acid β-N^4-(2-methylphenyl)piperazinamide, aspartic acid β-N^4-(3-methylphenyl)piperazinamide, aspartic acid β-N^4-(4-methylphenyl) piperazinamide, aspartic acid β-N^4-(2-methoxyphenyl)piperazinamide, aspartic acid β-N^4-(3-methoxyphenyl)piperazinamide, aspartic acid β-N^4-(4-methoxyphenyl)piperazinamide, aspartic acid β-N^4-(2-chlorophenyl) piperazinamide, aspartic acid β-N^4-(3-chlorophenyl)piperazinamide, aspartic acid β-N^4-(4-chlorophenyl)piperazin amide, aspartic acid β-N^4-(4-nitrophenyl)piperazinamide, aspartic acid β-N^4-(4-fluorophenyl) piperazinamide, aspartic acid β-N^4-(3-trifluoromethylphenyl)piperazinamide, aspartic acid β-N^4-(2,3-dimethylphenyl)piperazinamide, aspartic acid β-N^4-(2-pyridyl)piperazinamide, aspartic acid β-N^4-(2-pyrimidyl)piperazinamide, glutamic acid γ-methyl ester, glutamic acid γ-cyclopropyl ester, glutamic acid γ-benzyl ester and glutamic acid γ-phenethyl ester.

With respect to general formula [I], the parent α-amino acid for the α-amino acid residue represented by X or Y may be any isomer, whether D, L or DL, with preference given to the L-isomer for both X and Y.

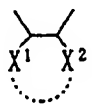
X preferably represents -Asp(R^1)-. -Asp(R^1)- is a group of the formula:



wherein R^1 represents a group represented by the formula:



wherein X^1 and X^2 independently represent a hydrogen atom, C_{1-6} alkyl group, C_{1-6} alkoxy group, a halogen atom or a nitro group, and X^1 and X^2 independently may be combined together to form a ring in

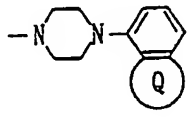


Examples of C_{1-6} alkyl group represented by X^1 and X^2 are methyl, ethyl, n-propyl, iso-propyl, n-butyl, isobutyl, tert-butyl, n-pentyl and n-hexyl, among which C_{1-3} alkyl group such as methyl, ethyl, n-propyl and isopropyl is preferred. Most preferred is methyl.

Examples of C_{1-6} alkoxy group represented by X^1 and X^2 are methoxy, ethoxy, n-propoxy, n-butoxy, n-pentyloxy and n-hexyloxy, among which C_{1-3} alkoxy group such as methoxy, ethoxy and n-propoxy is preferred. Most preferred is methoxy or ethoxy.

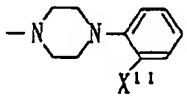
Examples of halogen atom represented by X^1 and X^2 are fluorine, chlorine, bromine and iodine, among which chlorine is preferred.

Examples of R^1 in case X^1 and X^2 are combined together to form a ring are represented by the formula;



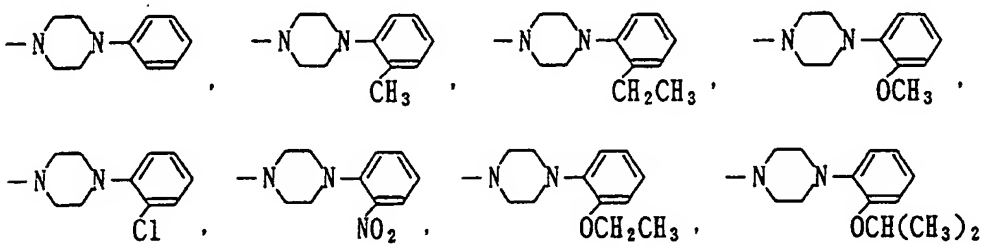
Examples of ring Q are 4- to 7-membered rings which may contain 1 to 3 hetero atom selected from O, N or S (e.g. saturated carbon rings, aromatic carbon rings, saturated heterocyclic rings and aromatic heterocyclic rings).

R^1 is preferably represented by the formula;



wherein X^{11} represents a hydrogen atom, C_{1-6} alkyl group, C_{1-6} alkoxy group, a halogen atom or a nitro group.

Preferred examples of R^1 are



Above mentioned -Asp(R^1)- may be any isomer, whether D, L or DL, with preference given to L-isomer.

With respect to general formula [I], the parent amino acid for the D-acidic-α-amino acid residue represented by A is exemplified by amino acids having an acidic group such as the carboxyl group, sulfo group or tetrazolyl group in the side chain thereof, including D-glutamic acid, D-aspartic acid, D-cysteic acid, D-homocysteic acid, D-β-(S-tetrazolyl)alanine and D-2-amino-4-(5-tetrazolyl)butyric acid, with preference given to D-glutamic acid, D-aspartic acid and D-cysteic acid.

With respect to general formula [I], the parent amino acid for the neutral-α-amino acid residue represented by B is exemplified by α-amino acids such as alanine, valine, norvaline, leucine, isoleucine, alloisoleucine, norleucine, tert-leucine, γ methylleucine, phenylglycine, phenylalanine, 1-naphthylalanine, 2-naphthylalanine, proline, 4-hydroxyproline, azetidine-2-carboxylic acid, pipecolic acid (piperidine-2-carboxylic acid), 2-thienylalanine, 2-thienylglycine, 3-thienylglycine, 1-aminocyclopropane-1-carboxylic acid, 1-aminocyclobutane-1-carboxylic acid, 1-aminocyclopentane-1-carboxylic acid, 1-aminocyclohexane-1-carboxylic acid, 1-aminocycloheptane-1-carboxylic acid, 2-cyclopentylglycine and 2-cyclohexylglycine. If the neutral-α-amino acid involves both the L- and D-configurations, the D-configuration is preferred. Greater preference is given to D-leucine, D-alloisoleucine, D-tert-leucine, D-γ methylleucine, D-phenylglycine, D-2-thienylalanine, D-2-thienylglycine, D-3-thienylglycine and D-2-cyclopentylglycine. The α-amino group of these neutral-α-amino acids may be replaced by a C_{1-6} alkyl group (e.g., methyl, ethyl, n-propyl or tert-butyl). Such α-amino acids include N-methylleucine, N-methylalloisoleucine, N-methyl tert-leucine, N-methyl γ methylleucine and N-methylphenylglycine, preferably of the D-configuration.

B preferably represents -NH-CHR^2-CO-, wherein R^2 represents C_{1-6} alkyl group, C_{3-7} cycloalkyl group, C_{3-7} cycloalkyl-C_{1-3} alkyl group, C_{1-6} alkylthio-C_{1-3} alkyl group, C_{3-7} cycloalkylthio-C_{1-3} alkyl group, C_{1-6} alkoxy-C_{1-3} alkyl group, C_{3-7} cycloalkoxy-C_{1-3} alkyl group, C_{1-6} alkylthio group, C_{3-7} cycloalkylthio group, C_{1-6} alkoxy group or C_{3-7} cycloalkoxy group.

Examples of C_{1-6} alkyl group represented by R^2 are methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl,

(1-methyl) propyl, tert-butyl, n-pentyl, (2-methyl) butyl, (3-methyl) butyl, neopentyl, n-hexyl, (2,2-dimethyl) butyl and (3,3-dimethyl) butyl, among which C_{4-6} alkyl group such as n-butyl, isobutyl, (1-methyl) propyl, tert-butyl, n-pentyl, (2-methyl) butyl, (3-methyl) butyl, (2-methyl) butyl, (3-methyl) butyl, neopentyl and n-hexyl is preferred.

Examples of C_{3-7} cycloalkyl group represented by R^2 are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl, among which C_{5-7} cycloalkyl group such as cyclopentyl, cyclohexyl and cycloheptyl is preferred.

Examples of C_{3-7} cycloalkyl-C_{1-3} alkyl group represented by R^2 are cyclopropylmethyl, cyclobutylmethyl, cyclobutylethyl, cyclobutylpropyl, cyclopentylmethyl, cyclopentylethyl, cyclopentylpropyl, cyclohexylmethyl, cyclohexylethyl, cyclohexylpropyl, cycloheptylmethyl and cycloheptylethyl, among which C_{3-7} cycloalkyl-methyl group such as cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, cycloheptylmethyl is preferred.

Examples of C_{1-6} alkylthio-C_{1-3} alkyl group represented by R^2 are methylthiomethyl, methylthioethyl, methylthiopropyl, ethylthiomethyl, ethylthioethyl, n-propylthiopropyl, iso-propylthiomethyl, n-butylthiomethyl, tert-butylthiomethyl, n-butylthioethyl, tert-butylthiopropyl and (1,1-dimethyl) propylthiomethyl, among which C_{3-7} alkylthio-methyl group such as iso-propylthiomethyl, n-butylthiomethyl, tert-butylthiomethyl and (1,1-dimethyl) propylthiomethyl is preferred.

Examples of C_{3-7} cycloalkylthio-C_{1-3} alkyl group represented by R^2 are cyclopropylthiomethyl, cyclopropylthioethyl, cyclopropylthiopropyl, cyclobutylthiomethyl, cyclobutylthioethyl, cyclobutylthiopropyl, cyclopentylthiomethyl, cyclopentylthioethyl, cyclohexythiomethyl and cycloheptylthiomethyl, among which C_{4-7} cycloalkylthiomethyl group such as cyclobutylthiomethyl, cyclopentylthiomethyl, cyclohexylthiomethyl and cycloheptylthiomethyl is preferred.

Examples of C_{1-6} alkoxy-C_{1-3} alkyl group represented by R^2 are methoxymethyl, methoxyethyl, methoxypropyl, ethoxymethyl, ethoxyethyl, n-propoxymethyl, n-propoxyethyl, iso-propoxymethyl, iso-propoxyethyl, n-butoxymethyl, n-butoxyethyl, tert-butoxymethyl, tert-butoxyethyl, n-pentyloxymethyl, n-pentyloxyethyl, (1,1-dimethyl) propoxymethyl, (1,1-dimethyl) propoxyethyl, n-hexyloxymethyl and n-hexyloxyethyl, among which C_{1-6} alkoxy-methyl group such as methoxymethyl, ethoxymethyl, n-propoxymethyl, iso-propoxymethyl, n-butoxymethyl, tert-butoxymethyl, n-pentyloxymethyl, (1,1-dimethyl) propoxymethyl and n-hexyloxymethyl is preferred. More preferred are iso-propoxymethyl, tert-butoxymethyl, (1,1-dimethyl) propoxymethyl and n-hexyloxymethyl.

Examples of C_{3-7} cycloalkoxy-C_{1-3} alkyl group represented by R^2 are cyclopropoxymethyl, cyclopropoxyethyl, cyclobutoxymethyl, cyclobutoxyethyl, cyclopentyloxymethyl, cyclopentyloxyethyl, cyclohexyloxymethyl and cycloheptyloxymethyl, among which C_{3-7} cycloalkoxy-methyl group such as cyclopropoxymethyl, cyclobutoxymethyl, cyclopentyloxymethyl, cyclohexyloxymethyl and cycloheptyloxymethyl is preferred.

Examples of C_{1-6} alkylthio group represented by R^2 are methylthio, ethylthio, n-propylthio, iso-propylthio, n-butylthio, tert-butylthio, n-pentylthio, (1,1-dimethyl) propylthio and n-hexylthio, among which C_{3-6} alkylthio group such as n-propylthio, iso-propylthio, n-butylthio, tert-butylthio, n-pentylthio, (1,1-dimethyl) propylthio and n-hexylthio is preferred.

Examples of C_{3-7} cycloalkylthio group represented by R^2 are cyclopropylthio, cyclobutylthio, cyclopentylthio, cyclohexylthio and cycloheptylthio, among which C_{4-7} cycloalkylthio group such as cyclobutylthio, cyclopentylthio, cyclohexylthio and cycloheptylthio is preferred.

Examples of C_{1-6} alkoxy group represented by R^2 are methoxy, ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, n-pentyloxy, (1,1-dimethyl) propoxy and n-hexyloxy, among which C_{3-6} alkoxy group such as n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, n-pentyloxy, (1,1-dimethyl) propoxy and n-hexyloxy is preferred.

Examples of C_{3-7} cycloalkoxy group represented by R^2 are cyclopropoxy, cyclobutoxy, cyclopentyloxy, cyclohexyloxy and cycloheptyloxy, among which C_{4-7} cycloalkoxy group such as cyclobutoxy, cyclopentyloxy, cyclohexyloxy and cycloheptyloxy is preferred.

R^2 is preferably C_{1-6} alkyl group, more preferably C_{4-6} alkyl group such as n-butyl, iso-butyl, (1-methyl) propyl, tert-butyl, n-pentyl, (2-methyl) butyl, (3-methyl) butyl, neopentyl and n-hexyl, with greater preference is given to tert-butyl and neopentyl.

Above mentioned α-amino acid represented by -NH-CHR^2-CO- may be any isomer, whether D, L or DL, with preference given to D-isomer.

With respect to general formula [I], the parent amino acid for the L-α-amino acid residue represented by C is exemplified by commonly known L-α-amino acids such as glycine, L-alanine, L-valine, L-norvaline, L-leucine, L-isoleucine, L-tert-leucine, L-norleucine, L-methionine, L-2-aminobutyric acid, L-serine, L-threonine, L-phenylalanine, L-aspartic acid, L-glutamic acid, L-asparagine, L-glutamine, L-lysine, L-tryptophan, L-arginine,

L-tyrosine and L-proline, with preference given to L-leucine, L-norleucine and L-tryptophan. The α-amino group of these L-α-amino acids may be replaced by a C_{1-6} alkyl group (e.g., methyl, ethyl, n-propyl or tert-butyl). Such L-α-amino acids include L-N-methylleucine, L-N-methylnorleucine and L-N(α)-methyltryptophan.

With respect to general formula [I], the parent amino acid for the D-α-amino acid residue having an aromatic cyclic group represented by E is exemplified by D-α-amino acids having an aromatic cyclic group in the side chain thereof. Examples of such amino acids include D-tryptophan, D-5-methyltryptophan, D-phenylalanine, D-tyrosine, D-1-naphthylalanine, D-2-naphthylalanine, D-3-benzothienylalanine, D-4-biphenylalanine and D-pentamethyl phenylalanine, with preference given to D-tryptophan and D-5-methyltryptophan. D-tryptophan is more preferred. The α-amino group of these D-α-amino acids having an aromatic ring may be replaced by a C_{1-6} alkyl group (e.g., methyl, ethyl, n-propyl or tert-butyl). The amino group of the indole ring of D-tryptophan may be replaced by a hydrocarbon group such as a C_{1-6} alkyl (e.g., methyl, ethyl, n-propyl or tert-butyl), C_{3-8} cycloalkyl (e.g., cyclopentyl or cyclohexyl), C_{6-12} aryl (e.g., phenyl or 4-methylphenyl) or C_{7-15} aralkyl (e.g., benzyl or phenethyl) or by an acyl group such as a C_{1-6} aliphatic acyl (e.g., formyl, acetyl or propionyl), C_{4-9} alicyclic acyl (e.g., cyclopentanecarbonyl or cyclohexanecarbonyl), C_{7-15} arylacyl (e.g., benzoyl or 4-methylbenzoyl), C_{8-16} aralkylacyl (e.g., phenylacetyl, 2-phenylpropionyl, 3-phenylpropionyl or diphenylacetyl) or C_{1-6} alkoxycarbonyl (e.g., methoxycarbonyl or ethoxycarbonyl). Such α-amino acids include D-N(α)-methyltryptophan, D-N-methylphenylalanine, D-N-methyltyrosine, D-N^{in}-methyltryptophan, D-N^{in}-ethyltryptophan, D-N^{in}-formyltryptophan and D-N^{in}-acetyltryptophan. D-N^{in}-methyltryptophan, D-N^{in}-formyltryptophan and D-N^{in}-acetyltryptophan are preferred.

E preferably represents Trp (N^{in}-R^3), wherein R^3 represents a hydrogen atom, C_{1-6} alkyl group, C_{3-7} cycloalkyl group, -COR^4 (R^4 represents a hydrogen atom, C_{1-6} alkyl group, C_{6-15} aryl group or C_{6-15} aryl-C_{1-3} alkyl group), -$COOR^5$ (R^5 represents C_{1-6} alkyl group, C_{6-15} aryl group or C_{6-15} aryl-C_{1-3} alkyl group) or -$CONHR^6$ (R^6 represents a hydrogen atom, C_{1-6} alkyl group, C_{6-15} aryl group or C_{6-15} aryl-C_{1-3} alkyl group) and R^3 is directly combined with N atom of indole group in tryptophan residue.

Examples of C_{1-6} alkyl group represented by R^3 are methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, (1-methyl) propyl, tert-butyl, n-pentyl, (2-methyl) butyl, (3-methyl) butyl, neopentyl, n-hexyl, (2,2-dimethyl) butyl and (3,3-dimethyl) butyl, among which C_{1-3} alkyl group such as methyl, ethyl, n-propyl and iso-propyl is preferred.

Examples of C_{3-7} cycloalkyl group represented by R^3 are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl, among which C_{5-7} cycloalkyl group such as cyclopentyl, cyclohexyl and cycloheptyl is preferred.

Examples of C_{1-6} alkyl group represented by R^4, R^5 and R^6 are methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, (1-methyl) propyl, tert-butyl, n-pentyl, (2-methyl) butyl, (3-methyl) butyl, neopentyl, n-hexyl, (2,2-dimethyl) butyl and (3,3-dimethyl) butyl, among which C_{1-3} alkyl group such as methyl, ethyl, n-propyl and iso-propyl is preferred.

Examples of C_{6-15} aryl group represented by R^4, R^5 and R^6 are phenyl, α-naphthyl and β-naphthyl, among which phenyl is preferred.

Examples of C_{6-15} aryl-C_{1-3} alkyl group represented by R^4, R^5 and R^6 are benzyl, phenylethyl, phenylpropyl, α-naphthylmethyl, α-naphthylethyl, α-naphthylpropyl, β-naphthylmethyl, β-naphthylethyl, β-naphthylpropyl, among which C_{6-15} aryl-methyl group such as benzyl, α-naphthylmethyl and β-naphthylmethyl, is preferred.

Specific embodiment of -COR^4 is exemplified by formyl, acetyl, propionyl, butyryl, isobutyryl, isovaleryl, pivaloyl, n-benzylcarbonyl, benzoyl and phenylacetyl.

Specific embodiment of -$COOR^5$ is exemplified by methoxycarbonyl, ethoxycarbonyl, phenoxycarbonyl and benzyloxycarbonyl.

Specific embodiment of -$CONHR^6$ is exemplified by carbamoyl, methylaminocarbonyl, ethylaminocarbonyl, n-propylaminocarbonyl, isopropylaminocarbonyl, n-butylaminocarbonyl, iso-butylaminocarbonyl, phenylaminocarbonyl and benzylaminocarbonyl.

R^3 is preferably a hydrogen atom and -COR^4 (R^4 represents a hydrogen atom, C_{1-6} alkyl group, C_{6-15} aryl group or C_{6-15} aryl-C_{1-3} alkyl group), with greater preference is given to a hydrogen atom, formyl and acetyl.

In the hexapeptide represented by general formula [I], or a salt thereof followings are preferred:

X is an L-isomer; Y is an L-isomer; A is selected from the group consisting of D-glutamic acid, D-aspartic acid, D-cysteic acid and D-tetrazolylalanine residues; B is of the D-configuration; B is selected from the group consisting of 1-aminocyclopropane-1-carboxylic acid, 1-aminocyclobutane-1-carboxylic acid, 1-aminocyclopentane-1-carboxylic acid, 1-aminocyclohexane-1-carboxylic acid and 1-aminocycloheptane-1-carboxylic acid; B is selected from the group consisting of D-leucine, D-alloisoleucine, D-tert-leucine, D-γ methyl leucine, D-phenylglycine, D-2-thienylglycine, D-3-thienylglycine, D-2-cyclopentylglycine, D-phenylalanine, D-2-thieny-

lalanine, D-valine, D-2-furylglycine and D-3-furylglycine residues; C is selected from the group consisting of L-leucine, L-isoleucine, L-valine, L-norleucine and L-α-amino acid residues having an aromatic group; E is selected from the group consisting of D-tryptophan or derivatives thereof, D-1-naphthylalanine, D-2-naphthylalanine, D-benzothienylalanine, D-4-bisphenylalanine and D-pentamethyl phenylalanine residues; the D-tryptophan derivative is selected from the group consisting of D-N^{In}-methyltryptophan, D-N^{In}-formyltryptophan and D-N^{In}-acetyltryptophan residues; More preferred ones are followings. A is a D-aspartic acid residue; X is a tryptophan, L-(β-4-phenylpiperazinamido)aspartic acid, L-[β-4-(2-methoxyphenyl)piperazinamid]aspartic acid, L-N(δ)-phenylacetylornithine(δ is a superscript, the same applies below), L-(N^4-[indol-3-yl]acetyl)ornithine, L-(4-benzyloxy)proline, L-(N^5-benzyl)glutamine or L-(N(δ)-[indol-3-yl]ethyl)asparagine residue; Y is an L-leucine, L-aspartic acid or L-O-benzylserine residue; B is a D-leucine, D-γ methyl leucine, D-2-thienylglycine or D-3-thienylglycine residue; C is selected from the group consisting of L-leucine, L-phenylalanine and L-tryptophan residues; and E is a D-tryptophan residue.

The anti-endothelin substance in the present invention is preferably the peptide(I) described in European Patent Publication No. 528312 and Japanese Patent Application No. 278722/1993.

Most preferably, the anti-endothelin substance is a peptide shown below.

(1) cyclo[-D-Asp-Asp(R1')-Asp-D-Thg(2)-Leu-D-Trp-],

(2) cyclo[-D-Asp(OC_2H_5)-Asp(R1')-Asp(OC_2H_5)-D-Thg(2)-Leu-D-Trp-]

(3) cyclo[-D-Asp-Asp(B7)-Asp-DγMeLeu-Leu-D-Trp-]

wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide; Thg(2) represents 2-thienylglycine; Leu represents leucine; Trp represents tryptophan; Asp(B7) represents aspartic acid β-4-(2-methoxyphenyl)piperazinamide; γMeLeu represents γ-methylleucine.

The above-described anti-endothelin substance, peptides in particular, may be used in the form of salts, preferably pharmacologically acceptable salts. Such salts may be organic or inorganic. Examples of the inorganic salts include salts with bases such as alkali metals (e.g., sodium and potassium), and polyvalent metals such as alkaline earth metals (e.g., calcium and magnesium), zinc, copper and aluminium and salts with inorganic acids (e.g., hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid and phosphoric acid). Examples of organic salts include salts with organic acids such as carboxylic acid (e.g., formic acid, acetic acid, trifluoroacetic acid and maleic acid), organic sulfonic acid (methanesulfonic acid, benzenesulfonic acid and toluenesulfonic acid) and amino acids (e.g., arginine, aspartic acid and glutamic acid), ammonium salts and salts with organic bases such as tert-amine (e.g., trimethylamine, triethylamine, pyridine, picoline, dicyclohexylamine and N-N'-dibenzylethylenediamine). When the anti-endothelin substance has an acidic group such as carboxyl, sodium salts and salts with arginine are preferred. When the anti-endothelin substance has a basic group such as amino, hydrochlorides and acetates are preferred.

The above-described salts may be in the form of complexes. Examples of the complexes are complexes with alkali metals (e.g., sodium and potassium) and polyvalent metals such as alkaline earth metals (e.g., calcium and magnesium), zinc, copper and aluminium. The complexes are preferably complexes with polyvalent metals such as alkaline earth metals (e.g., calcium and magnesium), zinc, copper and aluminium, with greater preference given to a zinc complex.

The peptide represented by the general formula:

$$\boxed{\text{A'-X-Y-B-C-E}} \qquad [II]$$

wherein A' represents a D-acidic-α-amino acid residue which is esterified with an alkyl group and other symbols have the same meanings as defined above, or a salt thereof, among the peptide [I], or ester thereof, or salt thereof, is novel.

In the D-acidic-α-amino acid residue which is esterified with an alkyl, represented by A', the alkyl group are exemplified by methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert-butyl, n-pentyl and n-hexyl, among which C_{1-3} alkyl group such as methyl, ethyl, n-propyl and iso-propyl is preferred.

The peptide [II] or salt thereof is produced by subjecting the peptide [I] or salt thereof to a per se known esterification with an alkyl group.

When sustained-release preparations are produced by using the peptide [II] or salt thereof, thus obtained sustained-release preparations exhibit both suppression of initial burst of drug and constant release of drug.

The zinc salt of a peptide represented by the general formula [I] is novel.

The zinc salt of a peptide [I] is produced by mixing the peptide [I] or a water-soluble salt (e.g.: sodium salt) thereof with a water-soluble zinc salt in water. The zinc salt of peptide [I] precipitated is isolated by centrifu-

gation and the like. Thus obtained precipitate is dispersed in distilled water and centrifuged again. These operations are repeated to give a purified zinc salt of peptide [I]. The purified zinc salt of peptide [I] is subjected to drying such as vacuum drying and lyophilizing. The mixing ratio (peptide [I]/water-soluble zinc salt) (mol ratio) is about 10/1 to 1/10, preferably about 5/1 to 1/5. The concentration of these in water is within solubility of each and not lower than solubility of a produced complex. The above-mentioned water-soluble zinc salts are exemplified by inorganic acid zinc salts such as zinc halogenide (e.g., zinc chloride, zinc bromide, zinc iodide, zinc fluoride), zinc sulfate, zinc nitrate and zinc thiocyanate and organic acid zinc salts such as aliphatic carboxylic acid zinc salts (e.g., zinc acetate, zinc glycolate, zinc lactate, zinc tartrate) and aromatic acid zinc salts (e.g., zinc benzoate, zinc salicylate, zinc phenolsulfate). The water-soluble zinc salts are preferably aliphatic carboxylic acid zinc salts, with greater preference given to zinc acetate.

When sustained-release preparations are produced by using the zinc salt of the peptide [I], thus obtained sustained-release preparations exhibit both suppression of initial burst of drug and constant release of drug. The sustained-release preparations are high in drug content.

In the present invention, examples of means which can be used for sustained retention of the anti-endothelin substance in the living body are injectable sustained-release preparations (e.g., microcapsules and microspheres) using a biodegradable polymer, and indwellable preparations (shaped as needles, for example). Also available are electrically driven pumps or osmotic pressure pumps (Alzet etc.) capable of sustained release of a given amount of anti-endothelin substance. Other examples include preparations for non-invasive administration at such sites as the skin (percutaneous preparations), mucosa (transnasal preparations, transvaginal preparations etc.) and digestive tract (oral preparations, rectal suppositories etc.). The sustained-release preparation mentioned herein may be any preparation, as long as the pharmaceutical action is sustained for at least 24 hours after a single administration or an almost constant effective blood concentration lasts for at least 24 hours, with preference given to a preparation capable of sustaining the pharmaceutical action or an effective blood concentration for at least 72 hours after a single administration. Although an effective blood concentration may be sustained by increasing the frequency of administration in the case of oral preparations of short duration of action, increased frequency of administration is inconvenient for the patient and the degree of certainty is low. Microcapsular preparations using a biodegradable polymer are preferred because they are easy to administer and long in duration of action after administration. The anti-endothelin substance incorporated in the microcapsular preparation is preferably an endothelin antagonist. Although the amount of endothelin antagonist added varies depending on the activity thereof, target disease, duration of effect and other factors, the endothelin antagonist is used at normally about 0.001 to 50% (w/w), preferably about 0.01 to 30% (w/w), and more preferably about 0.1 to 20% (w/w), relative to the base biodegradable polymer.

Examples of biodegradable polymers include aliphatic polyesters (e.g., polymers, copolymers or mixtures thereof produced from one or more of α-hydroxycarboxylic acids such as glycolic acid, lactic acid and hydroxybutyric acid, hydroxydicarboxylic acids such as malic acid, hydroxytricarboxylic acids such as citric acid and others), poly-α-cyanoacrylic acid esters, polyamino acids (e.g., poly-γ-benzyl-L-glutamic acid) and maleic anhydride copolymers (e.g., styrene-maleic acid copolymers). These may be used as a mixture. Here, the type of polymerization may be random, block or graft.

The biodegradable polymer is preferably an aliphatic polyester (e.g., a polymer, copolymer or mixture thereof produced from one or more α-hydroxycarboxylic acids as glycolic acid, lactic acid and hydroxybutyric acid, hydroxydicarboxylic acids such as malic acid, hydroxytricarboxylic acids such as citric acid, and others).

Examples of the above-described copolymers include copolymers of glycolic acid and other α-hydroxy acids, the α-hydroxy acid being preferably lactic acid, 2-hydroxybutyric acid or the like. Although the α-hydroxycarboxylic acid may be a D-, L- or D,L-isomer, it is preferable that the ratio of the D-isomer/L-isomer (mol%) fall within the range from about 75/25 to 25/75. More preferably, the α-hydroxycarboxylic acid is a hydroxycarboxylic acid wherein the ratio of D-isomer/L-isomer (mol%) falls within the range from about 60/40 to 40/60.

With respect to the copolymer of glycolic acid and 2-hydroxybutyric acid, it is preferable that glycolic acid account for about 10 to 75 mol% and 2-hydroxybutyric acid account for the remaining portion. More preferably, glycolic acid accounts for about 20 to 75 mol%, still more preferably about 30 to 70 mol%. The glycolic acid copolymer has a weight-average molecular weight of about 2,000 to 50,000, preferably about 3,000 to 40,000, more preferably about 8,000 to 25,000. The dispersity of the glycolic acid copolymer (weight-average molecular weight/number-average molecular weight) is preferably about 1.2 to 4.0. Greater preference is given to a copolymer wherein the dispersity is about 1.5 to 3.5. The present glycolic acid copolymer can be produced by a known process such as the method described in Japanese Patent Unexamined Publication No. 28521/1986. It is preferable that the copolymer be produced by catalyst-free dehydration polymerization condensation.

The above-described glycolic acid copolymer may be used in a mixture with polylactic acid. Although the polylactic acid may be a D-isomer, L-isomer or a mixture thereof, it is preferable that the ratio of the D-isomer/L-isomer (mol%) fall within the range from about 75/25 to 20/80. More preferred is a polylactic acid wherein the

ratio of the D-isomer/L-isomer (mol%) falls within the range from about 60/40 to 25/75, with greater preference given to a polylactic acid wherein the ratio of the D-isomer/L-isomer (mol%) falls within the range from about 55/45 to 25/75. The polylactic acid preferably has a weight-average molecular weight of about 1,500 to 30,000. More preferred is a polylactic acid wherein the weight-average molecular weight falls within the range from about 2,000 to 20,000, with greater preference given to a polylactic acid wherein the weight-average molecular weight falls within the range from about 3,000 to 15,000. Also, the dispersity of the polylactic acid is preferably about 1.2 to 4.0, more preferably about 1.5 to 3.5.

For producing polylactic acid, two methods are known: ring-opening polymerization of lactide, a dimer of lactic acid, and dehydration polymerization condensation of lactic acid. For obtaining a polylactic acid of relatively low molecular weight for the present invention, direct dehydration polymerization condensation of lactic acid is preferred. This method is, for example, described in Japanese Patent Unexamined Publication No. 28521/1986.

The present glycolic acid copolymer and polylactic acid are used over the mixing ratio range of, for example, from about 10/90 to 90/10 (% by weight), preferably from about 20/80 to 80/20, more preferably from about 30/70 to 70/30.

In the case of a copolymer of glycolic acid and lactic acid, the content ratio (lactic acid/glycolic acid) (mol%) is preferably about 100/0 to 40/60, more preferably about 90/10 to 45/55. The weight-average molecular weight of the copolymer of glycolic acid and lactic acid is preferably about 4,000 to 25,000, more preferably about 5,000 to 20,000.

The dispersity of the copolymer of glycolic acid and lactic acid (weight-average molecular weight/number average molecular weight) is preferably from about 1.2 to 4.0, more preferably from about 1.5 to 3.5. The copolymer of glycolic acid and lactic acid can be produced by a known method, such as the method described in Japanese Patent Unexamined Publication No.28521/1986. The copolymer is preferably produced by catalyst-free dehydration polymerization condensation.

In the present invention, the aliphatic polyester produced by catalyst-free dehydration polymerization condensation usually has a terminal carboxyl group.

More preferably, the biodegradable polymer is an aliphatic polyester (e.g., a polymer, copolymer or mixture thereof produced from one or more α-hydroxycarboxylic acids such as glycolic acid, lactic acid and hydroxybutyric acid, hydroxydicarboxylic acids such as malic acid, hydroxytricarboxylic acids such as citric acid, and others) as having a terminal carboxyl group.

A biodegradable polymer having a terminal carboxyl group is a polymer in which the number-average molecular weights by GPC determination and that by end-group determination almost agree.

To quantitate terminal free carboxyl groups, about 1 to 3 g of the biodegradable polymer is dissolved in a mixed solvent of acetone (25 ml) and methanol (5 ml), and the solution is quickly titrated with a 0.05 N alcoholic solution of potassium hydroxide while stirring at room temperature with phenolphthalein as an indicator to determine the terminal carboxyl group content; the number-average molecular weight is calculated from the following equation:

Number-average molecular weight by end-group determination = 20,000 A/B

where A is the weight mass (g) of the biodegradable polymer, and B is the amount (ml) of the 0.05 N alcoholic solution of potassium hydroxide added until the titration end point is reached.

This value is hereinafter referred to as number-average molecular weight by end-group determination.

For example, in the case of a polymer having a terminal carboxyl group, produced from one or more α-hydroxy acids by catalyst-free dehydration polymerization condensation, the number-average molecular weight by GPC determination and the number-average molecular weight by end-group determination almost agree with each other. On the other hand, in the case of a polymer having no terminal carboxyl groups and which is synthesized from a cyclic dimer by ring-opening polymerization using a catalyst, the number-average molecular weight by end-group determination is significantly higher than that by GPC determination. This difference makes it possible to clearly differentiate a polymer having a terminal carboxyl group from a polymer having no terminal carboxyl group.

While the number-average molecular weight by end-group determination is an absolute value, that by GPC determination is a relative value that varies depending on various analytical conditions (e.g., kind of mobile phase, kind of column, reference substance, slice width, baseline etc.); it is therefore difficult to have an absolute numerical representation of the latter. However, the description that the number-average molecular weights by GPC determination and end-group determination "almost agree" here denotes that the latter falls within the range from about 0.5 to 2 times, preferably from about 0.8 to 1.5 times, the former. Also, the description that the number-average molecular weight by end-group determination is "significantly higher" than the number-average molecular weight by GPC determination here denotes that the former is about 2 times or more greater than the latter.

In the present invention, preference is given to a polymer wherein the number-average molecular weights by GPC determination and by end-group determination almost agree.

Regarding weight-average molecular weights and number-average molecular weights by GPC determination, the present specification holds that the former is based on polystyrene obtained by gel permeation chromatography (GPC) with 9 polystyrenes as reference substances with weight-average molecular weights of 120,000, 52,000, 22,000, 9,200, 5,050, 2,950, 1,050, 580 and 162, respectively. Measurements were taken using a GPC column KF804Lx2 (produced by Showa Denko) and an RI monitor L-3300 (produced by Hitachi, Ltd.), with chloroform as a mobile phase.

The dispersity is calculated by the formula: (weight-average molecular weight/number-average molecular weight)

The sustained-release preparation of the present invention can, for example, be produced from a w/o emulsion with a solution containing an anti-endothelin substance as an internal aqueous phase and a solution containing a biodegradable polymer as an oil phase. This is achieved by known methods, including aqueous drying (in-water drying), phase separation, spray drying and modifications thereof.

The solvent used in the oil phase for the above-mentioned methods is preferably an organic solvent which dissolves biodegradable polymers and which has a boiling point not higher than 120°C. Such solvents include halogenated hydrocarbons (e.g., dichloromethane, chloroform and carbon tetrachloride), alcohols (e.g., ethanol and methanol) and acetonitrile. These may be used in combination. The solvent is preferably dichloromethane, acetonitrile or the like.

When the anti-endothelin substance for the present invention has a carboxyl group, its water solubility is low because it is often acidic; to increase its pharmaceutical solubility, it is often used in the form of an organic or inorganic salt. Such organic or inorganic salts are preferably alkali metal salts (e.g., sodium salt and potassium salt), with preference given to sodium salt. In order to include a pharmacologically necessary amount of drug, it is necessary to prepare a solution of very high concentration, however, since the volume of the aqueous phase for preparing the above w/o emulsion is usually very small. In such case, when the drug is low in water solubility, though soluble in water, it can fail to be completely dissolved, resulting in uneven mixing in preparing the emulsion. By dissolving the anti-endothelin substance along with an organic basic substance, a uniform solution of the anti-endothelin substance can be prepared that is soluble in water but low in solubility. Also, the addition of an organic basic substance suppresses the usually rapid initial drug release from microcapsules produced using a biodegradable polymer, allowing sustained release of a given amount of drug over a given period of time. The organic basic substance is preferably a basic amino acid, particularly arginine, histidine, lysine or the like. The organic basic substance is further exemplified by a peptide comprising two or more of basic amino acids such as arginyl-arginine.

As for the content ratio of the organic basic substance, the weight ratio of the anti-endothelin substance to the organic basic substance is normally 1:1,000 to 1,000:1, preferably 1:100 to 100:1, and more preferably 1:10 to 10:1. The weight ratio of the biodegradable polymer to the organic basic substance is normally 1,000:1 to 5:1, preferably 500:1 to 10:1, and more preferably 100:1 to 10:1.

In the process of producing sustained-release preparations, the addition of water-soluble polyvalent metal salts suppress the initial burst of drug, allowing sustained release of a given amount of drug over a given period of time and causing a high drug content. The water-soluble polyvalent metal salts may be any one, without limitation, as long as it is soluble in water and does not adversely affect the living body.

The water-soluble polyvalent metal salts are preferably polyvalent metal salts whose water solubility at normal temperature (about 20°C) is over about 20 mg/ml, more preferably over about 100 mg/ml.

Examples of water-soluble polyvalent metal salts are polyvalent metal salts with inorganic acids and that with organic acids. Polyvalent metals are exemplified by alkaline earth metal (e.g., calcium, magnesium), zinc (II), iron (II, III), copper (II), tin (II, IV) and aluminium (II, III). Inorganic acids are exemplified by hydrohalogenic acid (e.g., hydrochloric acid, hydrobromic acid, hydroiodic acid, hydrofluoric acid), sulfuric acid, nitric acid and thiocyanic acid. Organic acids are exemplified by aliphatic carboxylic acid (e.g., acetic acid, glycolic acid, lactic acid, tartaric acid) and aromatic acid (e.g., benzoic acid, salycylic acid, phenolsulfonic acid). The water-soluble polyvalent metal salts are preferably water-soluble zinc salts. The water-soluble zinc salts are exemplified by inorganic acid zinc salts such as zinc halogenide (e.g., zinc chloride, zinc bromide, zinc iodide, zinc fluoride), zinc sulfate, zinc nitrate and zinc thiocyanate and organic acid zinc salts such as aliphatic carboxylic acid zinc salts (e.g., zinc acetate, zinc glycolate, zinc lactate, zinc tartrate) and aromatic acid zinc salts (e.g., zinc benzoate, zinc salicylate, zinc phenolsulfate). The water-soluble zinc salts are preferably aliphatic carboxylic acid zinc salts, with greater preference given to zinc acetate.

As for the content ratio of the water-soluble polyvalent metal salts, the weight ratio of the anti-endothelin substance to the water-soluble polyvalent metal salts is preferably 1:100 to 100:1, more preferably 1:10 to 10:1. The weight ratio of the biodegradable polymer to the water-soluble polyvalent metal salts is preferably 1,000:1

to 1:1, more preferably 100:1 to 2:1.

In the present invention, the anti-endothelin substance may be dissolved or suspended directly in an organic solvent solution of the biodegradable polymer. The anti-endothelin substance may be soluble or insoluble in the organic solvent. The anti-endothelin substance is sometimes soluble in the solution of the biodegradable polymer in the organic solvent, even when the anti-endothelin substance is insoluble in the organic solvent. Any organic solvent is acceptable, as long as it is substantially immiscible with water and dissolves the biodegradable polymer, and the resulting polymer solution dissolves the anti-endothelin substance. The organic solvent preferably has a water solubility not higher than 3% at normal temperature (20°C). Also, the boiling point of the organic solvent is preferably not higher than 120°C. Example organic solvents include halogenated hydrocarbons (e.g., dichloromethane, chloroform, chloroethane, trichloroethane and carbon tetrachloride), ethers (e.g., isopropyl ether), fatty acid esters (e.g., butyl acetate) and aromatic hydrocarbons (e.g., benzene, toluene and xylene). These may be used in combination in appropriate ratios. The organic solvent is preferably dichloromethane. Dissolution of the anti-endothelin substance means that no anti-endothelin substance remains undissolved in the resulting solution, as examined by macroscopic observation at normal temperature (20°C).

The sustained-release preparation of the present invention is preferably produced by, for example, the method of microcapsulation (or modification thereof) based on aqueous drying or phase separation as described below.

(i) Aqueous drying method (w/o/w method)

An anti-endothelin substance is dissolved in water. The anti-endothelin substance concentration in the aqueous solution is, for example, about 0.1 to 500% (w/v), preferably about 1 to 400% (w/v), and more preferably about 10 to 300% (w/v). To the aqueous solution, an organic basic substance, preferably a basic amino acid (e.g., arginine) or a peptide comprising two or more of basic amino acids (e.g.,- arginyl-arginine) may be added. The concentration of the organic basic substance used for this purpose in the aqueous solution is about 0.01 to 500% (w/v), preferably about 0.1 to 400% (w/v), and more preferably about 1 to 300% (w/v). To the aqueous solution, water-soluble polyvalent metals may be added in the same manner as the organic basic substance. To the aqueous solution may be added pH regulators (e.g., acetic acid, hydrochloric acid and sodium hydroxide), stabilizers (e.g., serum albumin and gelatin), preservatives (paraoxybenzoic acids) and other additives. The aqueous solution thus obtained is emulsified and dispersed in an organic solvent solution of a biodegradable polymer or copolymer synthesized from α-hydroxycarboxylic acid to yield a w/o emulsion. Although the biodegradable polymer concentration in the organic solvent solution varies depending on the molecular weight of the biodegradable polymer and the kind of organic solvent, it is selected over the range from about 0.01 to 80% (w/w), preferably about 0.1 to 70% (w/w), and more preferably about 1 to 60% (w/w).

The ratio of the aqueous solution and the organic solvent solution of the biodegradable polymer is normally 1:1,000 (v/v) to 1:1 (v/v), preferably 1:100 (v/v) to 1:5 (v/v), and more preferably 1:50 (v/v) to 1:5 (v/v). This emulsification is achieved by known methods of dispersion using a turbine type mechanical stirrer, homogenizer etc.

The w/o emulsion thus prepared is added to an aqueous phase to form a w/o/w emulsion, followed by evaporation of the solvent in the oil phase, to yield microcapsules. The volume of the aqueous phase is chosen over the range normally from about 1 to 10,000 times, preferably from about 2 to 5,000 times, and more preferably from about 5 to 2,000 times the volume of the oil phase.

In addition to the above additives, an emulsifier may be added to the aqueous phase. The emulsifier may be any one, as long as it is capable of forming a stable o/w emulsion. Examples of such emulsifiers include anionic surfactants, nonionic surfactants, polyoxyethylene castor oil derivatives, polyvinylpyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin and hyaluronic acid. These may be used singly or in combination. The concentration of emulsifier used may be chosen as appropriate over the range normally from about 0.001 to 20% (w/w), preferably from about 0.01 to 10% (w/w), and more preferably from about 0.05 to 5% (w/w).

The microcapsules thus obtained are collected by centrifugation or filtration, after which they are repeatedly washed with distilled water in several cycles to remove the free anti-endothelin substance, emulsifier etc. adhering to the microcapsule surface, again dispersed in distilled water etc. and then lyophilized. Where necessary, the microcapsules are heated under reduced pressure to further remove the water and organic solvent. Preferably, this removal is carried out at a heating rate of 10 to 20°C per minute at a temperature higher by at least 5°C than the intermediate glass transition point of the biodegradable polymer, as determined using a differential scanning calorimeter, usually within 1 weeks or 2 or 3 days, more preferably within 24 hours, after the microcapsules have reached a given temperature.

(ii) Aqueous drying method (o/w method)

An anti-endothelin substance is added to an organic solvent solution of a biodegradable polymer to a ratio by weight as defined above, to prepare an organic solvent solution or suspension containing both the anti-endothelin substance and the biodegradable polymer. In this operation, the biodegradable polymer concentration in the organic solvent solution varies depending on the molecular weight of the biodegradable polymer and the kind of the organic solvent, it is chosen over the range normally from about 0.01 to 80% (w/w), preferably from about 0.1 to 70% (w/w), and more preferably from about 1 to 60% (w/w). To the organic solvent solution or suspension, water-soluble polyvalent salts may added.

The organic solvent solution or suspension thus prepared is added to an aqueous phase to form an o/w emulsion, followed by evaporation of the solvent in the oil phase, to yield microcapsules. The volume of the aqueous phase is chosen over the range normally from about 1 to 10,000 times, preferably from about 2 to 5,000 times, and more preferably from about 5 to 2,000 times the volume of the oil phase.

In addition to the above additives, an emulsifier may be added to the aqueous phase. The emulsifier may be any one, as long as it is capable of forming a stable o/w emulsion. Examples of such emulsifiers include anionic surfactants, nonionic surfactants, polyoxyethylene castor oil derivatives, polyvinylpyrrolidone, polyvinyl alcohol, carboxymethyl cellulose, lecithin, gelatin and hyaluronic acid. These may be used singly or in combination. The concentration of the emulsifier used may be chosen as appropriate over the range normally from about 0.001 to 20% (w/w), preferably from about 0.01 to 10% (w/w), and more preferably from about 0.05 to 5% (w/w).

The microcapsules thus obtained are collected by centrifugation or filtration, after which they are repeatedly washed with distilled water in several cycles to remove the free anti-endothelin substance, emulsifier etc. adhering to the microcapsule surface, and again dispersed in distilled water etc. and then lyophilized. Where necessary, the microcapsules are then heated under reduced pressure to further remove water and organic solvent. Preferably, this removal is achieved at a heating rate of 10 to 20°C per minute at a temperature higher by at least 5°C than the intermediate glass transition point of the biodegradable polymer, as determined using a differential scanning calorimeter, usually within 1 weeks or 2 or 3 days, more preferably within 24 hours after the microcapsules have reached a given temperature.

(iii) Phase separation method

In producing microcapsules by the phase separation method, a coacervating agent is gradually added to the above-described w/o emulsion or organic solvent solution during stirring, to separate and solidify the biodegradable polymer. The coacervating agent is added in an amount by volume about 0.01 to 1,000 times, preferably about 0.05 to 500 times, and more preferably about 0.1 to 200 times the volume of the w/o emulsion or organic solvent solution.

Any coacervating agent is acceptable, as long as it is a polymer, mineral oil or vegetable oil compound that is miscible in the solvent for the biodegradable polymer and which does not dissolve the polymer. Example coacervating agents include silicon oil, sesame oil, soybean oil, corn oil, cotton seed oil, coconut oil, linseed oil, mineral oil, n-hexane and n-heptane. These may be used in combination.

The microcapsules thus obtained are collected by filtration, after which they are repeatedly washed with heptane etc. to remove the coacervating agent. The free drug and solvent are removed in the same manner as in the aqueous drying method.

Solvent removal can be achieved by known methods, including the method in which the solvent is evaporated under normal or gradually reduced pressure during stirring using a propeller stirrer or magnetic stirrer, and the method in which the solvent is evaporated while adjusting the degree of vacuum using a rotary evaporator etc.

In production by the aqueous drying method or coacervation method, an antiflocculant may be added to prevent grain flocculation. The antiflocculant is exemplified by water-soluble polysaccharides such as mannitol, lactose, glucose and starches (e.g., corn starch), proteins such as glycine, fibrin and collagen and inorganic salts such as sodium chloride and sodium hydrogen phosphate.

In producing microcapsules by the spray drying method, a w/o emulsion or organic solvent solution containing the above-described anti-endothelin substance and biodegradable polymer is sprayed via a nozzle into the drying chamber of a spray drier to volatilize the organic solvent in the fine droplets in a very short time to yield fine microcapsules. The nozzle is exemplified by the double-fluid nozzle, pressure nozzle and rotary disc nozzle. Where desired, to prevent microcapsule flocculation, an aqueous solution of the above-described antiflocculant may be effectively sprayed via another nozzle simultaneously with spraying of the w/o emulsion or organic solvent solution containing the anti-endothelin substance and biodegradable polymer.

The microcapsules thus obtained may have their water and organic solvent removed at increased temperature under reduced pressure as necessary.

The above-described microcapsules can be administered as such or in the form of various dosage forms of non-oral preparations (e.g., intramuscular, subcutaneous or visceral injections or indwellable preparations, nasal, rectal or uterine transmucosal preparations) or oral preparations (e.g., capsules such as hard capsules and soft capsules), or solid preparations such as granules and powders or liquid preparations such as syrups, emulsions and suspensions.

In addition to the above-described dosage forms of microcapsules, the w/o emulsion or organic solvent solution containing an anti-endothelin substance and biodegradable polymer can be shaped in rods, needles, pellets, films and other forms and administered as intramuscular, subcutaneous or visceral injections or indwellable preparations, nasal, rectal or uterine transmucosal preparations, oral preparations (e.g., capsules such as hard capsules and soft capsules), solid preparations such as granules and powders, and liquid preparations such as syrups, emulsions and suspensions.

The injectable preparation of the present invention can be produced by known methods. The injectable preparation is produced by, for example, suspending the above-described sustained-release preparation of microcapsules etc. in water, along with a dispersing agent (e.g., surfactants such as Tween 80 and HCO-60, and polysaccharides such as carboxymethyl cellulose and sodium alginate), a preservative (e.g., methyl paraben and propyl paraben), an isotonizing agent (e.g., sodium chloride, mannitol, sorbitol and glucose), to yield an aqueous suspension, or by dispersion in a vegetable oil such as sesame oil or corn oil or middle-chain fatty acid triglyceride (e.g., Migriol 812) to yield an oily suspension. The particle size of the sustained-release preparation is chosen over the range from about 0.1 to 300 μm, for instance, as long as the requirements concerning the degree of dispersion and needle passage are met, when the sustained-release preparation is used as an injectable suspension. Preferably, the particle size falls within the range from about 1 to 150 μm, more preferably from about 2 to 100 μm. A sustained-release preparation can be prepared as a sterile preparation without limitation by the method in which the entire production process is sterile, the method in which a gamma ray is used as a sterilant, and the method in which an antiseptic is added.

With low toxicity, the sustained-release preparation of the present invention can be safely used in mammals (e.g., humans, bovines, swines, dogs, cats, mice, rats and rabbits).

The sustained-release preparation of the present invention is used to treat or prevent endothelin-associated diseases, particularly chronic ones. Such diseases include cardiac/cerebral circulatory diseases, renal diseases, hypertension (e.g., pulmonary hypertension), asthma, inflammation, arthritis, hepatic cancer, cirrhosis and chronic complications in diabetes mellitus. The sustained-release preparation of the present invention is used to treat or prevent arteriosclerosis, diabetic nephropathy, diabetic myocarditis and diabetic retinopathy, in particular.

Varying depending on type, content and dosage form of the active ingredient anti-endothelin substance, duration of anti-endothelin substance release, target disease (e.g., diabetic nephropathy), subject animal and other factors, the dose of the sustained-release preparation may be set at levels such that the anti-endothelin substance is effective. The dose per administration of the active ingredient anti-endothelin substance is chosen as appropriate over the range from about 0.01 to 100 mg/kg body weight for each adult when the preparation is a 1-month preparation. More preferably, the dose may be chosen as appropriate over the range from about 0.05 to 50 mg/kg body weight.

The dose per administration of the sustained-release preparation is chosen as appropriate over the range from about 0.1 to 1,000 mg/kg body weight for each adult. More preferably, the dose may be chosen as appropriate over the range from about 0.5 to 500 mg/kg body weight. Dosing frequency can be chosen as appropriate, e.g., once weekly, once every several weeks, once monthly or once every several months, depending on type, content and dosage form of the active ingredient anti-endothelin substance, duration of anti-endothelin substance release, subject disease, subject animal and other factors.

The preparation of the present invention may be used in combination with other drugs, specifically conventional therapeutic drugs for diabetic nephropathy, such as hypotensive drugs. Although the preparation of the present invention may be stored at normal temperatures or cold places, it is preferable to store it at a cold place. Normal temperatures and cold places mentioned herein are as defined by the Pharmacopoeia of Japan.

The present invention is hereinafter described in more detail by means of the following working examples and experimental examples, which are not to be construed as limitative.

Reference Example 1

Synthesis of cyclo[-D-Asp-Asp(B7)-Asp-D-γMeLeu-Leu-D-Trp-] disodium salt

4.4 g of cyclo[-D-Asp-Asp(B7)-Asp-D-γMeLeu-Leu-D-Trp-] (hereinafter referred to briefly as peptide B)

was dissolved in 50 ml of methanol and concentrated. The concentrate was again dissolved in 50 ml of methanol and subjected to ice cooling. To thus obtained solution 0.1 N NaOH solution (46.4 ml) was added dropwise, and the pH of the solution was adjusted to 7-8 by further addition of 0.1 N NaOH solution. The resulting solution was concentrated. The concentrate was lyophilized after addition of distilled water. Peptide B disodium salt (Yield 4.5 g)

Elemental analysis: As $C_{47}H_{61}N_9O_{11}Na_2 \cdot CF_3CO_2Na \cdot 0.5CH_3CO_2Na \cdot 3H_2O$			
Calculated:	C, 49.18;	H, 5.65;	N, 10.32
Found:	C, 49.08;	H, 5.50;	N, 10.33

Example 1

51 mg of the disodium salt of the cyclic peptide
cyclo[-D-Asp-Asp(R1')-Asp-D-Thg(2)-Leu-D-Trp-]
wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide; Thg(2) represents 2-thienylglycine; Leu represents leucine; Trp represents tryptophan, described in European Patent Publication No. 528312 and 49 mg of L-arginine (Wako Pure Chemical) were dissolved in 300 ul of distilled water. This solution was added to a solution of 1.92 g of a lactic acid-glycolic acid copolymer (lactic acid/glycolic acid = 75/25 mol%, GPC weight-average molecular weight 14,000, GPC number-average molecular weight 2,000, number-average molecular weight by end-group determination 2,200, produced by Wako Pure Chemical Industry, Lot No. 920729) in 2 ml of dichloromethane, and the mixture was stirred using a homogenizer (Polytron) to yield a w/o emulsion. After cooling to 17°C, the emulsion was injected to 1,000 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry, Co., Ltd.), previously adjusted to 16°C, followed by stirring in a turbine homomixer at 7,000 rpm to yield a w/o/w emulsion, which was then stirred at room temperature for 3 hours to volatilize the dichloromethane and solidify the oil phase, which was then collected via centrifugation at 2,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). The precipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After the collected microcapsules were re-dispersed in a small amount of distilled water, 0.3 g of D-mannitol was added, and the dispersion was lyophilized to yield powdery microcapsules.

Example 2

About 39 mg of microcapsules as obtained in Example 1 was dispersed in 1.95 ml of a dispersant for injection (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol, all dissolved therein) to yield an injectable preparation.

Example 3

3.6 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid=75/25 mol %, GPC weight-average molecular weight 15,038, GPC number-average molecular weight 5,195, produced by Wako Pure Chemical Industry) was dissolved in 6.6 g (5 ml) of dichloromethane. To this solution was added a solution of peptide A disodium salt (250 mg) and L-arginine (100 mg) in 0.5 ml of distilled water, and the mixture was stirred for about 30 seconds using a homogenizer (Polytron) to yield a w/o emulsion. The emulsion was injected to 800 ml of a 0.1 % (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry, Co., Ltd.), previously adjusted to 18°C, followed by stirring in a turbine homomixer at 6,000 rpm to yield a w/o/w emulsion, which was then stirred at a room temperature for 3 hours to volatile the dichloromethane and solidify the oil phase, which was then collected via centrifugation at 2,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). The precipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After 100 mg of D-mannitol was added to the collected microcapsules, the microcapsules were re-dispersed in a small amount of distilled water, and the dispersion was lyophilized to yield powdery microcapsules.

The microcapsules thus obtained were homogenized and extracted in 0.1 M ammonium acetate solution containing 30% (v/v) acetonitrile for 3 hours, and then assayed by HPLC (high performance liquid chromatography).

As the result, the content of peptide A disodium salt was 5.2 mg per 100 mg of microcapsules.

Example 4

Powdery microcapsules were obtained in the same manner as Example 3, except that L-arginyl-arginine (Kokusan Chemical Works Ltd.) was substituted for L-arginine.

The content of peptide A disodium salt was 7.4 mg per 100 mg of microcapsules.

Example 5

Synthesis of cyclo[-D-Asp(OC_2H_5)-Asp(R1')-Asp(OC_2H_5)-D-Thg(2)-Leu-D-Trp-]

10 ml of ethanol was cooled to -10°C in a dry ice-acetone bath, and 2.6 ml of thionyl chloride was added in a small amount. After 5 minutes, 1.0 g of peptide A disodium salt was added to the mixture and stirred at room temperature. After 2 hours, ethanol and excess thionyl chloride was removed under reduced pressure to give an oily substance. The oily substance was dissolved in a small amount of ethanol, and again the solvent was removed under reduced pressure. This operation was repeated three times and a small amount of diethylether was added to give 1.05 g of titled compound. The result of analysis of peptide A diethylester was described below.

1) Mass spectrometry (LSIMS method):

$[M + H]^+ = 984$ (theoretical value = 984)

$[M + Na]^+ = 1,006$ (theoretical value = 1,006)

2)

Elemental analysis: As $C_{49}H_{61}N_9O_{11}S \cdot 2NaC1 \cdot 2H_2O \cdot HCl$			
Calculated:	C, 50.20;	H, 5.67;	N, 10.75
Found:	C, 50.35;	H, 5.75;	N, 10.81

Example 6

0.5 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid=50/50 ml %, GPC weight-average molecular weight 5,900, GPC number-average molecular weight 2,600, produced by Wako Pure Chemical Industry) was dissolved in 6.6 g (5 ml) of dichloromethane. To this solution was added 0.15 g of peptide A diethylester which was obtained in Example 5, and the mixture was stirred for about 30 seconds using a homogenizer (Polytron) to yield a s/o emulsion. The emulsion was injected to 400 ml of a 0.1 % (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry, Co., Ltd.), previously adjusted to 18°C, followed by stirring in a turbine homomixer at 6,000 rpm to yield a s/o/w emulsion, which was then stirred at a room temperature for 3 hours to volatile the dichloromethane and solidify the oil phase, which was then collected via centrifugation at 2,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). The precipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After 50 mg of D-mannitol was added to the collected microcapsules, the microcapsules were re-dispersed in a small amount of distilled water, and the dispersion was lyophilized to yield powdery microcapsules.

The microcapsules thus obtained were homogenized and extracted in 0.1 M phosphate buffered solution containing 50 % acetinitrile for 3 hours, and then assayed by HPLC (high performance liquid chromatography).

As the result, the content of peptide A diethylester was 25.2 mg per 100 mg of microcapsules.

Example 7

3.2 g of peptide A disodium salt and 7.28 g of zinc acetate di-hydrate were each dissolved in 160 ml of distilled water, and thus obtained two solutions were mixed together. This mixture was stayed at 4°C for a day, and then centrifuged at 3,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). Thus obtained precipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After small amount of distilled water was added to the collected precipitate to re-disperse the precipitate, the dispersion was lyophilized to yield a crude peptide A zinc salt as a 2.81 g of dried powder.

The dried powder thus obtained were homogenized and extracted in 50 mM EDTA solution containing 30 % (v/v) acetonitrile for 3 hours, and then assayed by HPLC (high performance liquid chromatography).

As the result, the content of peptide A in dried powder was 80.7% (w/w).

Example 8

0.97 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid= 75/25 mol %, GPC weight-average molecular weight 15,038, GPC number-average molecuar weight 5,195, produced by Wako Pure Chemical Industry) was dissolved in 13.2 g (10 ml) of dichloromethane. To this solution was added the crude peptide A zinc salt (300 mg) which was obtained in Example 7, and the mixture was stirred for about 30 seconds using a homogenizer (Polytron) to yield a s/o emulsion. The emulsion was injected to 400 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry, Co., Ltd.), previously adjusted to 18°C, followed by stirring in a turbine homomixer at 6,000 rpm to yield a s/o/w emulsion, which was then stirred at a room temperature for 3 hours to volatile the dichloromethane and solidify the oil phase, which was then collected via centrifugation at 2,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). The precipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After 50 mg of D-mannitol was added to the collected microcapsules, the microcapsules were re-dispersed in a small amount of distilled water, and the dispersion was lyophilized to yield powdery microcapsules.

The microcapsules thus obtained were homogenized and extracted in 50 mM EDTA (ethylenediaminetetraacetic acid) solution containing 30% (v/v) acetonitrile for 3 hours, and then assayed by HPLC (high performance liquid chromatography).

As the result, the content of a crude peptide A zinc salt in terms of peptide A disodium salt was 21.2 mg per 100 mg of microcapsules.

Example 9

Powdery microcapsules were obtained in the same manner as Example 3, except that peptide B disodium salt was substituted for peptide A disodium salt.

The content of peptide B disodium salt was 5.2 mg per 100 mg of microcapsules.

Example 10

1.2 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid= 75/25 mol %, GPC weight-average molecular weight 13,585, GPC number-average molecular weight 4,413, produced by Wako Pure Chemical Industry) was dissolved in 26.4 g (20 ml) of dichloromethane. To this solution was added a solution of peptide A disodium salt (400 mg) and zinc acetate dihydrate (400 mg) in 1.7 ml of distilled water, and the mixture was stirred for about 30 seconds using a homogenizer (Polytron) to yield a w/o emulsion. The emulsion was injected to 800 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry, Co., Ltd.), previously adjusted to 18°C, followed by stirring in a turbine homomixer at 6,000 rpm to yield a w/o/w emulsion, which was then stirred at a room temperature for 3 hours to volatile the dichloromethane and solidify the oil phase, which was then collected via centrifugation at 2,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). The precipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After 50 mg of D-mannitol was added to the collected microcapsules, the microcapsules were re-dispersed in a small amount of distilled water, and the dispersion was lyophilized to yield powdery microcapsules.

The microcapsules thus obtained were homogenized and extracted in 50 mM EDTA solution containing 30% (v/v) acetonitrile for 3 hours, and then assayed by HPLC (high performance liquid chromatography).

As the result, the content of peptide A disodium salt was 12 mg per 100 mg of microcapsules.

Example 11

1.4 g of lactic acid-glycolic acid copolymer (lactic acid/glycolic acid= 75/25 mol %, GPC weight-average molecular weight 13,585, GPC number-average molecular weight 4,413, produced by Wako Pure Chemical Industry) was dissolved in 6.6 g (5 ml) of dichloromethane. To this solution was added peptide A disodium salt (437 mg) and zinc acetate di-hydrate (467 mg), and the mixture was stirred for about 30 seconds using a homogenizer (Polytron) to yield a s/o emulsion. The emulsion was injected to 800 ml of a 0.1% (w/w) aqueous solution of polyvinyl alcohol (EG-40, produced by The Nippon Synthetic Chemical Industry, Co., Ltd.), previously adjusted to 18°C, followed by stirring in a turbine homomixer at 6,000 rpm to yield a s/o/w emulsion, which was then stirred at a room temperature for 3 hours to volatile the dichloromethane and solidify the oil phase, which was then collected via centrifugation at 2,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). The pre-

cipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After 50 mg of D-mannitol was added to the collected microcapsules, the microcapsules were re-dispersed in a small amount of distilled water, and the dispersion was lyophilized to yield powdery microcapsules.

The microcapsules thus obtained were homogenized and extracted in 50 mM EDTA solution containing 30% (v/v) acetonitrile for 3 hours, and then assayed by HPLC (high performance liquid chromatography).

As the result, the content of peptide A disodium salt was 12.2 mg per 100 mg of microcapsules.

Comparative Example 1

Powdery microcapsules were obtained in the same manner as Example 3, except that peptide A disodium salt was not used.

Comparative Example 2

1.6 g of peptide A disodium salt and 3.65 g of zinc acetate were each dissolved in 80 ml of distilled water, and thus obtained two solutions were mixed together. This mixture was centrifuged at 3,000 rpm using a centrifuge (05PR-22, Hitachi, Ltd.). Thus obtained precipitate was again dispersed in distilled water, centrifuged and washed to remove the free drug etc. After a small amount of distilled water was added to the collected precipitate to re-disperse the precipitate, the dispersion was lyophilized to yield a crude peptide A zinc salt as 1.23 g of a dried powder.

Experimental Example 1

An injectable preparation as obtained in Example 2 was subcutaneously administered to the back of 8-week-old male SD rats. After administration, rats were killed at given intervals and the microcapsules remaining at the administration site were taken out and assayed for drug content. This procedure was repeated to obtain the time course of drug release from the microcapsules given to the live body. The results are shown in Figure 1. The drug content in the microcapsules given to the live body decreased over a period of 1 month or more, demonstrating that the anti-endothelin substance could be sustained in the live body.

Experimental Example 2

About 100 mg of microcapsules as obtained in Example 1 were dispersed in 2.5 ml of a dispersant for injection (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol, all dissolved therein). The resulting dispersion was subcutaneously administered to the back of 13-week-old male Wistar fatty rats. The male Wistar fatty rat, a line of rat which genetically develops obesity and hyperglycemia, is characterized by increased leakage of protein and albumin in urine with the development of hyperglycemia. The results of urinary protein and albumin assays in a control group receiving no microcapsules and an administration group receiving the microcapsule are given in Table 1.

Table 1

Urinary Albumin (mg/day,mean)					
Weeks after administration	0	2	4	6	8
Control group	9	-	32	-	37
Administration group	6	-	18	-	23
Urinary Protein (mg/day,mean)					
Weeks after administration	0	2	4	6	8
Control group	97	98	83	112	132
Administration group	98	68	68	64	92

As seen in Table 1, during the period of about 6 weeks after microcapsule administration, smaller amounts of protein and albumin were excreted in the urine, in comparison with the initial values and control values. These

results demonstrate that urinary protein and albumin excretion, a symptom of diabetic nephropathy, were suppressed during the period when the endothelin antagonist was sustained in the live body as shown in Experimental Example 1, suggesting the utility of the present invention as a therapy for diabetic nephropathy.

Experimental Example 3

About 190 mg of microcapsules as obtained in Example 3 were dispersed in 1.5 ml of dispersant for injection (distilled water containing 7.5 mg of carboxymethyl cellulose, 1.5 mg of polysorbate 80 and 75 mg of mannitol, all dissolved therein). The resulting dispersion was subcutaneously administered to the back of 8-week-old male Wistar fatty rats using 18 G needles (the dosage of peptide A disodium salt per one rat was about 10 mg). The same administration was conducted once a month for 3 months. As a control, microcapsules containing no peptide A disodium salt as obtained in Comparative Example 1 were subcutaneously administered to the back of 8-week-old male Wistar fatty rats.

At regular intervals after administration, excreted urine was sampled and urinary albumin was assayed. As seen in Table 2, 9 and 12 weeks after the administration, the urinary albumin excretion in an administration group receiving the microcapsules of Example 2 was suppressed, compared with that in a control group.

Table 2

Urinary Albumin (mg/day,mean)			
Weeks after administration	0	9	12
Control group	2±1	32±8	45±10
Administration group	3±1	17±4	26±11

Experimental Example 4

6-week-old male Wistar rats anaesthetized with pentobarbital were innoculated with 25 mg of deoxycorticosterone after surgical removal of lefthand side kidney. The rats were allowed to drink 1% (w/v) of saline solution freely for 3 weeks. The microcapsules as obtained in Example 3 were dispersed in a dispersant for injection (2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol, all dissolved in 0.5 ml of distilled water) to get 100 mg/ml of peptide A disodium salt in the resulting dispersion. The dispersion was subcutaneously administered to the back of the rats using 18 G needles (the dosage of peptide A disodium salt was 100 mg/kg). As a control, microcapsules containing no peptide A disodium salt as obtained in Comparative Example 1 were subcutaneously administered to the back of 6-week-old male Wistar rats which were treated in the same manner.

As the results, systolic pressure in an administration group receiving the microcapsules of Example 3 began to decrease at one week after administration, and was kept lower by about 28 and 25 mmHg compared with that in a control group each until 2 and 4 weeks after administration. These results demonstrate that the sustained release of anti-endothelin substance makes it possible to keep blood pressure low.

Experimental Example 5

5-week-old male Wistar rats surgically innoculated with Mini Osmotic Pump (Alzet Model 2002, produced by Alza) containing 45 mg of peptide A disodium salt were subcutaneously administered with monocrotaline (100 mg/kg). The Mini Osmotic Pump were replaced after 2 weeks. The release rate of peptide A disodium salt from the Mini Osmotic Pump was 2.5 mg/rat/day, calculated from the remaining amount of peptide A disodium salt in the removed Mini Osmotic Pump. As a control, Mini Osmotic Pump containing no peptide A disodium salt were surgically innoculated in 5-week-old male Wistar rats, and the rats were treated in the same manner. At 4 weeks after monocrotaline administration, chests of the rats anaesthetized with pentobarbital were surgically opened under artificial respiration, and the pressure of right ventricles was monitored via an inserted catheter after a steady stae was achieved.

As the results, in the group treated with peptide A disodium salt, elevation of right ventricle pressure was moderately suppressed (lower by 26 mmHg compared with a control group). In addition, hypertrophy of right ventricle was not significant (lower by 0.23 mg tissue/g body weight) compared with the control group. These results demonstrate that the sustained presence of endothelin antagonist in blood is effective enough to im-

prove the pathology of pulmonary hypertension, and that sustained release preparation of anti-endothelin substance is useful in the treatment of pulmonary hypertension.

Experimental Example 6

About 30 mg of the microcapsules as obtained in Example 6 were dispersed in 0.5 ml of a dispersant for injection (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol, all dissolved therein). The resulting dispersion was subcutaneously administered to the back of 9-week-old SD rats using 20 G needles (the dosage of peptide A diethylester per one rat was about 12.6 mg). At regular intervals after administration, blood was gathered from rats tails and the concentration of peptide A diethylester in serum was assayed by EIA (Enzyme immunoassay). As seen in Table 3 almost constant blood concentration was kept for 2 weeks.

Table 3

peptide A diethylester in serum (ng/ml)			
Days after administration	1	7	4
Administration group	17.8	19.7	12.5

Experimental Example 7

About 50 mg of the microcapsules as obtained in Example 8 were dispersed in 0.5 ml of a dispersant for injection (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol, all dissolved therein). The resulting dispersion was subcutaneously administered to the back of 6-week-old SD rats using 20 G needles (the dosage of a crude peptide A zinc salt per one rat was about 10 mg in terms of peptide A disodium salt). At regular intervals after administration, blood was gathered from rats tails and the concentration of peptide A in serum was assayed by EIA. The results are given in Table 4. The amount of a peptide A zinc salt in the table is calculated in terms of peptide A disodium salt.

Table 4

peptide A zinc salt in serum (ng/ml)				
Days after administration	1	7	14	21
Administration group	5.09	6.50	10.18	11.23

As seen in Table 4, an almost constant blood concentration was kept for 3 weeks in an administration group receiving the preparation of Example 7. As a control, a crude peptide A zinc salt as obtained in Comparative Example 2 was dispersed in the dispersant for injection and was subcutaneously administered to rats (the dosage of a crude peptide A zinc salt per one rat was about 10 mg in terms of peptide A disodium salt), then peptide A in serum was decreased to be undetectable 3 days after the administration.

Experimental Example 8

About 70 mg of the microcapsules as obtained in Example 10 were dispersed in 0.5 ml of a dispersant for injection (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol, all dissolved therein). The resulting dispersion was subcutaneously administered to the back of 6-week-old SD rats using 20 G needles (the dosage of peptide A disodium salt per one rat was about 10 mg). At regular intervals after administration, blood was gathered from rats tails and the concentration of peptide A disodium salt in serum was assayed by EIA. The results are given in Table 5.

Table 5

peptide A disodium salt in serum (ng/ml)				
Days after administration	1	7	14	21
Administration group	11.12	26.77	8.37	5.74

As seen in Table 5, an almost constant blood concentration was kept for 2 weeks in an administration group receiving the preparation of Example 10.

Experimental Example 9

About 70 mg of the microcapsules as obtained in Example 11 were dispersed in 0.5 ml of a dispersant for injection (distilled water containing 2.5 mg of carboxymethyl cellulose, 0.5 mg of polysorbate 80 and 25 mg of mannitol, all dissolved therein). The resulting dispersion was subcutaneously administered to the back of 6-week-old SD rats using 20 G needles (the dosage of peptide A disodium salt per one rat was about 10 mg). At regular intervals after administration, blood was gathered from rats tails and the concentration of peptide A disodium salt in serum was assayed by EIA. The results are given in Table 6.

Table 6

peptide A disodium salt in serum (ng/ml)			
Days after administration	1	7	14
Administration group	5.79	8.99	10.91

As seen in Table 6, an almost constant blood concentration was kept for 2 weeks in an administration group receiving the preparation of Example 11.

The sustained-release preparation of the present invention sustainedly releases an anti-endothelin substance, serving well in the treatment of endothelin-associated diseases, particularly chronic complications in diabetes mellitus.

[Brief description of the drawings]

Figure 1 shows the changes over time in percent drug retention in microcapsules of a sustained-release preparation given to rats, obtained at the site of administration in Experimental Example 1.

Claims

1. A sustained-release preparation which comprises an anti-endothelin substance and a biodegradable polymer.

2. The sustained-release preparation according to claim 1, wherein the anti-endothelin substance is an endothelin antagonist.

3. The sustained-release preparation according to claim 2, wherein the endothelin antagonist is a peptide.

4. The sustained-release preparation according to claim 2, wherein the endothelin antagonist is a peptide of the general formula:

$$\text{A-X-Y-B-C-E}$$

wherein X and Y independently represent an α-amino acid residue; A represents a D-acidic-α-amino acid residue; B represents a neutral-α-amino acid residue; C represents an L-α-amino acid residue; E represents a D-α-amino acid residue having an aromatic cyclic group, or an ester thereof, or a salt thereof.

5. The sustained-release preparation according to claim 4, wherein the peptide is a compound of the formula cyclo[-D-Asp-Asp(R1')-Asp-D-Thg(2)-Leu-D-Trp-] wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide; Thg(2) represents 2-thienylglycine; Leu represents leucine; and Trp represents tryptophan.

6. The sustained-release preparation according to claim 4, wherein A is a D-acidic-α-amino acid residue which is esterified with an alkyl group.

7. The sustained-release preparation according to claim 4, wherein Y is a L-acidic-α-amino acid residue.

8. The sustained-release preparation according to claim 4, wherein Y is a L-acidic-α-amino acid residue which is esterified with an alkyl group.

9. The sustained-release preparation according to claim 4, wherein the peptide is a compound of the formula, cyclo-[-D-Asp(OC_2H_5)-Asp(R1')-Asp(OC_2H_5)-D-Thg(2)-Leu-D-Trp-] wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide; Thg(2) represents 2-thienylglycine; Leu represents leucine; and Trp represents tryptophan.

10. The sustained-release preparation according to claim 4, wherein the salt is a polyvalent metal salt.

11. The sustained-release preparation according to claim 10, wherein the polyvalent metal salt is a zinc salt.

12. The sustained-release preparation according to claim 1, wherein the biodegradable polymer is an aliphatic polyester.

13. The sustained-release preparation according to claim 12, wherein the aliphatic polyester is a copolymer of glycolic acid and lactic acid.

14. The sustained-release preparation according to claim 13, wherein the copolymer has a weight-average molecular weight of about 2,000 to 50,000, as determined by Gel Permeation Chromatography.

15. The sustained-release preparation according to claim 13, wherein the copolymer has a dispersity of about 0.2 to 4.0.

16. The sustained-release preparation according to claim 1, which further comprises an organic basic substance.

17. The sustained-release preparation according to claim 1, which further comprises a water-soluble polyvalent metal salt.

18. The sustained-release preparation according to claim 1 for treatment of diseases caused by endothelin.

19. The sustained-release preparation according to claim 18, wherein the diseases are chronic diseases.

20. The sustained-release preparation according to claim 19, wherein the chronic diseases are chronic complications in diabetes mellitus.

21. The sustained-release preparation according to claim 20, wherein the chronic complications are diabetic nephropathy.

22. An injectable preparation which comprises the sustained-release preparation as claimed in claim 1.

23. A peptide of the general formula:

$$\text{A'-X-Y-B-C-E}$$

wherein X and Y independently represent an α-amino acid residue; A' represents a D-acidic-α-amino acid residue which is esterified with an alkyl group; B represents a neutral-α-amino acid residue; C represents an L-α-amino acid residue; E represents a D-α-amino acid residue having an aromatic cyclic group, or a

salt thereof.

24. The peptide according to claim 23, wherein X is an L-isomer.

25. The peptide according to claim 23, wherein Y is an L-isomer.

26. The peptide according to claim 23, wherein A' is D-glutamic acid or D-aspartic acid which is esterified with an alkyl group.

27. The peptide according to claim 23, wherein B is an D-isomer.

28. The peptide according to claim 23, wherein B is selected from the group consisting of D-leucine, D-alloisoleucine, D-tertiary leucine, D-gamma methyl leucine, D-phenylglycine, D-2-thienylglycine, D-3-thienylglycine, D-2-cyclopentylglycine, D-phenylalanine, D-2-thienylalanine, D-valine, D-2-furylglycine and D-3-furylglycine residues.

29. The peptide according to claim 23, wherein C is selected from the group consisting of L-leucine, L-phenylalanine and L-tryptophan residues.

30. The peptide according to claim 23, wherein E is selected from the group consisting of D-tryptophan or derivatives thereof, D-1-naphthylalanine, D-2-naphthylalanine, D-benzothienylalanine, D-4-bisphenylalanine and D-pentamethyl phenylalanine residues.

31. The peptide according to claim 23, wherein Y is an α-amino acid residue having a carboxyl group which is esterified with an alkyl group.

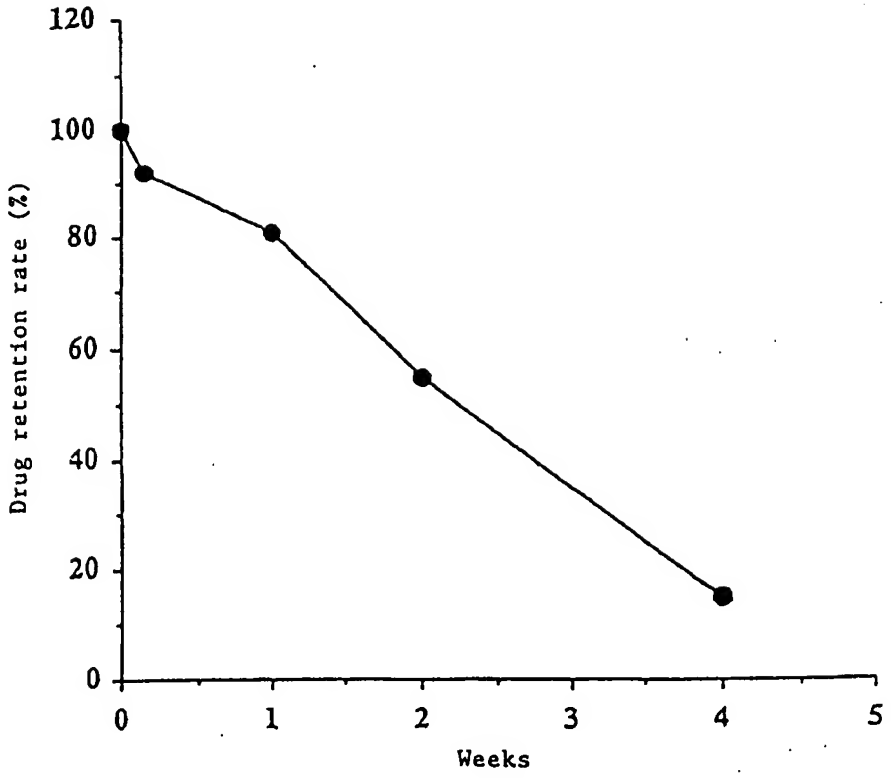
32. A peptide of the formula: cyclo-[-D-Asp(OC_2H_5)-Asp(R1')-Asp(OC_2H_5)-D-Thg(2)-Leu-D-Trp-], wherein Asp represents aspartic acid; Asp(R1') represents aspartic acid β-4-phenylpiperazinamide; Thg(2) represents 2-thienylglycine; Leu represents leucine; and Trp represents tryptophan, or a salt thereof.

33. A zinc salt of a peptide represented by the general formula:

$$\text{A-X-Y-B-C-E}$$

wherein X and Y independently represent an α-amino acid residue; A represents a D-acidic-α-amino acid residue; B represents a neutral-α-amino acid residue; C represents an L-α-amino acid residue; E represents a D-α-amino acid residue having an aromatic cyclic group.

Figure 1



European Patent Office

EUROPEAN SEARCH REPORT

Application Number

DOCUMENTS CONSIDERED TO BE RELEVANT

EP 94304566.6

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y,D	EP - A - 0 528 312 (TAKEDA CHEMICAL INDUSTRIES LTD.) * Claims 1-5, 7-10, 12-15,18 * --	1-5,7, 9,22-30,32	A 61 K 38/03 C 07 K 7/64 C 07 K 4/00
Y,P	EP - A - 0 601 799 (TAKEDA CHEMICA INDUSTRIES LTD.) * Claims 1,6-8,11-13 * ----	1,13-15	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

A 61 K
C 07 K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
VIENNA	06-10-1994	BÖHM

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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